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THE ACHROMATIC SPINDLE IN THE SPORE MOTHER CELLS OF OSMUNDA REGALIS.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. XXIII.

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(WITH PLATE XXII)

THE cytology of the vascular cryptogams has received scant attention at the hands of botanists, as a glance at the literature of the subject will suffice to show. Calkins (**6**) has described the divisions in the spore mother cells of Pteris and Adiantum, reaching the conclusion that in these plants there is a true qualitative reduction of the chromosomes, in Weismann's sense. Quite recently Calkins' view has been combated by Stevens (**14**), who studied the spore mother cells of Scolopendrium, Cystopteris, and Pteris. Osterhout's (**11**) article on Equisetum is memorable as the first complete account of the formation of a spindle uncontrolled by directive spheres or centrosomes. He found that the appearance of the spindle was heralded by a felt-work of threads about the nucleus, and that the threads later arranged themselves into a multipolar spindle, and afterwards into one characteristically bipolar. These three publications, together with that by Shaw (**12**) on fertilization in Onoclea, and those by Belajeff (**2, 3, 4**) and Shaw (**13**) which treat of the origin of the cilia of spermatozoids, make up almost the total of minute research into the cytology of the pteridophytes since the introduction of improved methods of technique.

Impelled by the meagerness of our knowledge, and by the want of accord among such papers as have been published on this subject, the writer two years ago began collecting and examining the sporangia of Lycopodium, Selaginella, and various ferns, to discover some of the forms most favorable for cytological investigation. None was found so good as Osmunda. All three of the species occurring in the northern United States were collected, but only *O. regalis* was found to be fixed and preserved in a condition suitable for the purpose, and it alone has received any considerable attention. For want of time a full examination of the reduction divisions was not possible, and therefore attention was centered only upon such features as it was hoped might be followed in detail in the time at disposal. Accordingly in this paper only the origin, structure, and fate of the achromatic spindle are to be considered, together with such comments upon the changes in the chromatin as seem necessary for clearness. A more extensive account of the chromatic elements of the spindle is reserved for a future occasion, after there has been opportunity for further study.

The methods of fixation, embedding, and staining require a brief mention. As fixing agents, chromo-acetic acid (1 per cent. chromic acid, 0.75 per cent. acetic acid), and Flemming's weaker solution were employed. Chloroform was used as the medium of transfer to paraffin. The sections were cut 5 μ or 10 μ in thickness. To differentiate the fibrillar structures most clearly, various combinations of the following stains were tried: nigrosin, Delafield's haematoxylin, erythrosin, iron-alum-haematoxylin, acid fuchsin, methyl-green, iodine-green, safranin, gentian-violet, orange G. Of these stains the two combinations which have yielded the best results are iodine-green and acid-fuchsin, which was found especially advantageous in dealing with the chromatic elements, and safranin and gentian-violet, which was most effective in bringing out the details of the achromatic figure.

The sporangia of Osmunda make their first appearance in the latter part of summer, and continue their growth in the autumn months. By mid autumn those of *O. cinnamomea* and

O. Claytoniana have reached the mother cell stage, in which condition they lie dormant during the winter. The division into spores takes place in the spring, usually in northern Indiana between April 15 and May 1. *O. regalis* does not reach so advanced a condition before winter as do the other species. Cell divisions in the sporogenous tissue occur in the spring, and it is not till after the middle of April that the mother cells are differentiated; the division into spores is effected about three weeks later.

A more exact statement will be instructive. April 6, material collected showed occasional karyokinetic figures in the sporangia. April 21, first collection in which no divisions were found in the sporogenous cells; the tapetal cells were in active multiplication. May 5, many nuclei were in synapsis, the first seen. May 10, most of the nuclei were in synapsis, but some in first division. May 13, in most cases both divisions were completed, and often no trace of the spindle remained.[1] This indicates that the resting and early spirem stages last for about two weeks, and the synapsis stage for three or four days; and that the two divisions are effected in quick succession within a period of two or three days. The difficulty of obtaining the various stages of the second division also affords evidence that the second division follows quickly after the first, and is completed in a much shorter time.

The resting mother cell (*fig. 1*) is angular in form, and presents a rather large nucleus. The cytoplasm is evenly distributed as a delicate network of stainable matter with irregular swellings at the points of intersection; it cannot be regarded as having the structure of foam. The nucleus has a structure similar to that of the cytoplasm, differing only in the greater coarseness of its meshes, the larger size of its granular thickenings, and its more intense reactions with stains. Two or three nucleoli are usually present. In the cytoplasm are numerous deeply staining granules of variable size, each usually lying in an unstained area, as if the granules had suffered contraction in

[1] It should be added that the collection of May 13 was not from the same locality as those of the earlier dates.

the process of fixation. Of the nature and function of these granules, which in staining properties resemble nucleoli, little was learned except that they are not starch. They are not peculiar to any phase of cell division, though rather more numerous in the resting mother cell, or the prophase. They cannot be extra-nuclear nucleoli, for there is no increase or diminution in their size and number contemporaneous with the dissolution or renewal of the nucleoli. Moreover, the nuclear membrane is yet unbroken, when, in the later prophase of division, the nucleoli disappear from view, and therefore, if the nucleolar substance passes out into the cytoplasm at this time, it must do so by osmosis, and any relation between it and the extra-nuclear granules must be conjectural. To the inquiry if they might not be the source, at least in part, of the material of the achromatic spindle, no satisfactory answer can be given; it may be so, but positive evidence is lacking.

The mother cells enlarge for about two weeks after they have reached their full number, gradually separating from one another and becoming more and more rounded in outline. The nuclei also grow larger and pass first into a spirem condition in which the chromatic ribbon is not a single thread but much branched and anastomosed, and later into synapsis. The details of these changes need not be considered in this paper.

Meanwhile, the cytoplasm has retained its reticulate structure. But towards the end of synapsis a change is discernible, and this change is the first indication of the future spindle. It begins as an aggregation of material immediately about the nucleus, causing this region to stain more deeply with gentian-violet. The structure of this accumulation of cytoplasm, when it is first recognizable, is so delicate as absolutely to baffle the powers of the microscope; nothing definite can be said with regard to it, except that it appears to be very finely granular, especially in preparations fixed in Flemming's solution. To a similar layer about the nucleus of the pollen mother cells of *Cobaea scandens*, Lawson (**8**) proposed to give the name perikaryoplasm, in virtue of its position. Since Strasburger's term

kinoplasm has the advantage of more general acceptance, it will be employed here to designate not only this differentiated layer of cytoplasm but also the spindle forming material in all its modifications.

As the kinoplasm increases in quantity, it begins to assume a somewhat definite outline and texture. It becomes distinctly granular, and the granules are often disposed in short rows which run nearly concentric with the periphery of the nucleus, but are intertangled more or less confusedly (*figs. 2, 4*). In cells which have been fixed in chromo-acetic acid a more distinct fibrous appearance is presented. In some cases the fibers appear to form a loose mat; in others they are so related as to resemble a delicate meshwork with the meshes flattened towards the nucleus. Though the appearance is such as to suggest that the fibers and meshes are only a modification of the cytoplasmic reticulum, the writer was unable to trace the steps of such a transformation. The kinoplasmic material, both in its earliest form and in that assumed after cell division before its final re-transformation into reticulate cytoplasm, could not be distinguished as either reticular or fibrillar, but was very indefinitely granular.

The reader will not fail to discover the similarity of these conditions to those described by Osterhout (**11**), as the preparatory steps of spindle formation in the spore mother cells of Equisetum, although in Equisetum no layer of granular matter was observed. But the similarity stops here. The spindle in Osmunda does not pass through a multipolar stage, nor is there at any time a zone of radiating fibers about the nucleus, such as were seen by Osterhout in Equisetum, and by Belajeff (**1**), Mottier (**9**), Lawson (**8**), and others in the pollen mother cells of various seed plants. Tripolar spindles, though occasionally met with (*fig. 10*), were of so rare occurrence that their beginning or fate could not be traced, and it is certain they are not normal stages in the development of the spindle.

The changes in the outline of the kinoplasmic mass are very simple and easy to follow. When it has become distinctly fibrillar in structure its fibers, all the time increasing in quantity at

the expense of the outer cytoplasm, begin to collect in greater abundance at opposite sides of the nucleus and to be pushed up into dome shaped prominences. This condition is very well shown in *fig. 3*. The threads of granules run nearly tangential to the nucleus, extending out some distance at the side, and it is obvious even at this time that the axis and the bipolarity of the spindle are already determined. It is rarely the case, however, that both poles are equally prominent from the beginning; commonly one develops considerably in advance of the other. A curious relation between the position of the chromatin in synapsis and the first formed pole of the spindle was observed so frequently that it cannot be regarded as accidental. In elongated cells in which the nucleus is situated excentrically, the first pole of the spindle is formed on that side of the nucleus where there is the greatest amount of cytoplasm, while in synapsis the chromatin was almost invariably observed to be gathered within the nucleus to the side farthest from the center of the cell; that is, the chromatin in synapsis and the kinoplasm are gathered towards opposite ends of the cell. Such a relation between chromatin and kinoplasm may be true of all the mother cells, but it can be traced only in those cells which have the distinction of a long and a short axis, since the polarity of the spindle is not apparent until some time subsequent to synapsis. These peculiarities will be referred to again when the second division is considered. Every care was taken to avoid the illusion into which an observer might be misled by the effects of irregularity of infiltration of the fixing or other reagents, and the conclusion was reached that these relations have a real existence and significance in the living cell.

The writer inadvertently had an excellent opportunity to examine a condition of the cell somewhat resembling synapsis. Finding that the young sporangia, which are ensheathed in a close covering of hairs, did not sink readily in the killing fluid, he first moistened some of them for a moment with alcohol. They sank immediately, but afterwards turned out to be quite unfit for study. It was perfectly easy to trace the path of the

invading alcohol. Both cytoplasm and nucleoplasm were pushed forward; but no one could mistake such a condition for synapsis if he had once become acquainted with the latter. Contrary to what might be looked for, it was the resting cell which suffered most by this treatment; the spindle fibers, the chromosomes, and the spirem thread were not seriously affected.

It will now be necessary to refer briefly to intranuclear changes. The chromatic material, emerging from synapsis, gradually unrolls and extends itself within the nuclear cavity into a much coiled spirem, apparently of one continuous thread, for no ends can be seen in uncut parts of the nucleus. The spirem shortens and thickens, and after a time is segmented into long irregular chromosomes, which continue the shortening and thickening process already begun in the spirem. It is easy to see that many of the chromosomes are split longitudinally into pairs, and the two parts of a pair are often twisted loosely about each other. They take up a peripheral position in the nucleus, being apparently in close contact with the nuclear membrane, The halves usually remain attached to each other for a time, giving rise to Xs and Ys, or loops (as in *fig. 5*), according to the mode of attachment. But while the shortening is still going on, some of the pairs fall apart; otherwise how can the number of chromosomes shown in *fig. 7* be accounted for, if Strasburger's (**15**) estimate of twelve[2] be correct? This number has been counted in a few cases in uncut nuclei of the age shown in *fig. 5*. and also in polar views of the late anaphase in which twelve daughter chromosomes have been seen symmetrically grouped about the pole. But in nuclei of the condition shown in *fig. 7* the number of separate chromatin masses is quite variable, fourteen, fifteen, or sixteen being most common, and even as many as twenty have been counted.[3] If these numbers indicate a falling apart of some of the chromosome pairs, there must be a subsequent reunion before their arrangement into such an equatorial plate as that shown in *fig. 8*, in which of the twelve chromatic

[2] See note at the end of the paper.

[3] Guignard counted as many as twenty-two.

masses eleven are clearly double. Whether in the case of the larger numbers all the groups represent pairs, or whether some of them are single chromosomes, has not been determined. It is by no means certain, however, that the number of chromosomes in the equatorial plate is so constant as theories of nuclear division which are accepted at the present time require us to assume.

There is a corresponding development of the spindle fibers coincident with the maturation of the chromosomes. The spindle acquires a more distinctly bipolar form, and its fibers, which become coarser and longer, run continuously from pole to pole. During the prophase they have the appearance of knotted cords or strings of loose beads. These features can be seen in *fig.* 5, and also in *fig.* 6, which represents a section of a cell cut sufficiently deep to remove one chromosome and part of the equatorial region of the spindle. But in the metaphase and anaphase the knotted appearance is no longer recognizable. The fibers then appear as stout uniform threads or rods (*figs.* 9, 10). The same statement can be made of the second division (*figs.* 17, 18); that is to say, the fibers at the time when they are functioning in the separation of the chromosomes are of uniform diameter and texture. They are rows of granules at all other times, either when they are disappearing or in process of formation.

It is evident that the achromatic spindle is wholly of cytoplasmic origin. If any nuclear material takes part in its formation, it can do so only after passing through the nuclear membrane. There can be no direct union of linin or other nuclear substance with the kinoplasm, for in nuclei, as far advanced in the prophase as those shown in *figs.* 5 and 7, the chromosomes are the only stainable constituent remaining; no fibers of any kind can be made out, nor any trace of the nucleolus.

A comparison of the shape of the spindle, as seen just before and after the dissolution of the nuclear membrane, suggests that this period is marked by a sudden change. While the nuclear membrane is still present, the spindle has its poles

rounded and ill defined, and its breadth relatively great in comparison with its length. But in all cells from which the nuclear membrane has disappeared, the spindle is seen to be considerably narrower, and at the same time longer and more sharply pointed. To explain these phenomena it seems necessary to suppose that a pressure, exerted by the spindle fibers upon the nucleus, is sustained by the nuclear membrane, and that when the membrane finally gives way, its collapse is attended by a sudden diminution of the diameter of the spindle (*figs. 7, 8*) together with a corresponding increase in length. At the same time, the pressure of the fibers crowds the chromosomes close together. If the fibers be conceived of as curved elastic rods, this action becomes intelligible; that they are rigid enough to exert pressure is probable from the fact that the second spindles frequently cause a widening of the ends of the mother cell, pushing out the wall so as to give it the form in section of a figure 8.

One effect of the sudden collapse of the nucleus is that the chromosomes, which were all necessarily on the inner side of the spindle fibers, are most of them forced to the outside. No distinction of central and mantle fibers was possible, though very great care was exercised at this point of the investigation. It is clear from *fig. 9* that the original fibers, which run meridionally, still remain so after the separation of the chromosomes. Repeated searching under a magnification of 2250 diameters failed to reveal any fibers other than those which run from pole to pole, or any thickening of the fibers towards the poles, as if some portion attached to the chromosome were undergoing contraction. How then are the chromosomes propelled? Inasmuch as there are no fibers discoverable by which they can be pulled, is it not possible that they have a power of motion in themselves?

This hypothesis is not absurd. It is quite as reasonable to assume an automobility of the chromosomes as a contractility of the spindle fibers. The initiatory separation of the chromosomes into pairs by longitudinal fission implies a power of movement

which is entirely independent of external tension; and the same inference is possible from the gradual shortening of the spirem to form the chromosomes, and from the re-expansion of the chromatin in the daughter nuclei.

Meanwhile, the outer region of the cytoplasm loses its reticulate structure (still evident in *figs. 3, 4*), and passing through a condition in which it appears to be a diffusely staining mass becomes converted into granules, as in *figs. 8* and *9*. This change, which is comparable to that preceding the organization of the kinoplasm into fibrillae, advances gradually from the spindle outwards towards the cell wall. Directly after the metaphase the granules of this outer zone arrange themselves into rows stretching from the poles out towards the equatorial region of the cell. This condition is shown in *fig. 9*, and again in the second division in *figs. 17* and *18*. These rows of granules, which soon become more threadlike, will be spoken of as *secondary* fibers, to distinguish them from the primary fibers that form the central framework of the spindle. They are not mantle fibers, for they do not enter into connection with the chromosomes, and are only just coming into existence when the latter begin to withdraw from the equatorial plate towards the poles. The secondary fibers multiply rapidly, and by the end of the anaphase fill all the outer part of the cell; in fact all the cytoplasm seems to have been exhausted in producing them.

Almost immediately there begins a breaking down of the spindle fibers, indicated in the first place by their reassuming the appearance of dotted threads. In all cases the primary spindle is the first to disappear; then the secondary fibers break down and all the stainable cytoplasm is of granular texture (*figs. 11, 13*). A cell plate is first formed, which however comes to nothing; it can be traced through *figs. 12–18, 21*.

Though the second division follows quickly after the first, there is a sufficient interval between to allow the formation of a nuclear membrane and the partial reorganization of the chromatin. The chromosomes after assuming a very symmetrical pattern about the poles unite into a chromatic mass which

takes the shape of a shallow cup with the concavity towards the center of the cell.

If there is any synapsis in the second division, it is represented by the case of *fig. 13*. It will be interesting to compare this with the conditions prevailing at synapsis of the mother cell. In *fig. 13*, as in the synapsis formerly described, the chromatin is bunched on the side of the cell most remote from the greater mass of kinoplasm. In this case it is easy to understand how these conditions have arisen from those preexisting. Whether the relation of the chromatin to the kinoplasm is determined in the mother cell in the same manner, by the position of the last preceding spindle, it is impossible to say, on account of the long rest which the mother cell passes through. If it is so determined, it argues strongly for the organic continuity of the kinoplasm, or at least of some specific substance which retains a definite position in the cell and reacts on the cytoplasm so as to cause it, at the proper time, to be transformed into a fibrillar texture and organize the spindle. It should be remembered, however, that the first visible signs of approaching division in the mother cell are intranuclear, hence so far as we can judge from appearances, if the stimulus to division originates from some special region or structure, and not from the activity of the cell as a unit, we must assume that the primary impetus proceeds from the chromatin.

Apparently the preparations within the nucleus for the second division, and those in the cytoplasm, do not always progress with equal rapidity relatively to each other, for one may meet with cases in which the second spindles are well formed, while the nuclear membrane is still unbroken, and others in which the nuclear membrane is gone and the chromosomes of the second division well organized, while the spindle is still very imperfect. Invariably, however, the development of the second spindle is preceded by an accumulation of material along those borders of the daughter nuclei which were in contact with the old spindle. The fibers first appear in this region also, and the spindle as seen in sections across this part of the cell is bipolar from the

beginning. The poles are not diametrically opposite each other, but both lie on one side of the nucleus, as shown in *fig. 15*[1]. Invariably, too, the axes of the second spindle are at right angles to that of the first; that is, though they may be at any angle with reference to each other, they always lie in planes which are parallel to the first cell plate (*figs. 15–18*). In the preparations selected for drawing, the second spindles are either parallel (*figs. 14–17, 21*), or at right angles (*fig. 18*). It must not be understood, however, that these cases are typical in this respect; they were chosen because in them both the spindles are in view at once.

Undoubtedly the material used for the building up of the second spindle is obtained from the disintegration of the first, not as fibers, however, but as granules; and in this abundance of granular matter ready formed in the cell, we may perhaps see an explanation of the rapidity of the second division.

The phenomena attending the metaphase of the second divisions agree completely with those of the first division. There is the same absence of mantle fibers (*figs. 17, 18*), the same continuity of the fibers from pole to pole (*fig. 19*), the same excess of fibers over the number of chromosomes (*figs. 18, 19*). In the anaphase there is the same elongation of the chromosomes into crooked or lobulated rods, the same beaded appearance of the fibers as soon as the chromosomes have passed to the poles (*fig 21*), and the same development of secondary fibers which run from the poles towards the equator.

The beginning of the secondary fibers is shown in *fig. 19;* they are more abundant and longer in *figs. 20* and *21*, in which it is seen that those put out from the poles of one spindle meet those from the other. The secondary fibers so meeting unite by their ends into continuous threads, which connect the four poles of the primary spindles. In this way four secondary spindles are formed; and thus the four daughter nuclei are now joined by six symmetrically placed spindles, of which two are primary and four secondary. Meantime, there has been a slight rotation of the primary spindles so that their planes, which were at

first parallel to one another, come to lie at an angle of 60°. This results in placing the four daughter nuclei equidistant from one another in the tetrahedral arrangement which is characteristic of fern spores. Whether the rotation is brought about by the action of the secondary fibers, or by some other influence acting on the nuclei, could not be determined. In *fig. 21* two primary spindles are shown, and some of the seconday fibers not yet united by their extremities. In *fig. 22* are one primary spindle (that on the left side) and two secondary; the axes of the other three spindles would make angles of 60° with these or with the plane of the paper.

The disappearance of the spindle has been followed with great care in order to discover, if possible, in what manner the kinoplasm is metamorphosed into ordinary cytoplasm. It was found that the middle fibers of all the spindles are the first to disintegrate, the material being used up in part to build the cell plate. After the spindles are no longer distinguishable, a considerable amount of granular or amorphous matter remains on the inner side of each nucleus (*fig. 24*); finally this disappears and the cytoplasm assumes a reticulate structure throughout (*fig. 25*). All attempts to identify the granules of kinoplasm with microsomata, or the fibrous matter of the spindle with the cytoplasmic reticulations, were unsuccessful. The fibers of the spindle are composed of modified cytoplasm, which in the transformation loses its characteristic structure and becomes first structureless in appearance, then granular, then fibrous, and in returning to its normal condition reverses these steps.

SUMMARY.

The achromatic spindle originates wholly from cytoplasmic material (kinoplasm) which accumulates about the nucleus in the synapsis or spirem stage in the form of an indefinitely granular mass of stainable matter.

The kinoplasm becomes distinctly granular; then the granules arrange themselves into short rows concentric with the nuclear membrane; finally the rows of granules are massed in

greatest abundance on opposite sides of the nucleus, foreshadowing the development of a bipolar spindle.

Usually one pole is formed considerably in advance of the other; and in cells cut parallel to their long axis, it can be seen that the first pole (the greatest accumulation of kinoplasm) is on the side of the nucleus remote from the chromatic mass of synapsis.

The spindle is bipolar from the beginning. Němec's (**10**) generalization, therefore, that sporogenous cells as compared with vegetative cells are characterized by their spindles passing through a multipolar phase, does not hold good of Osmunda.

The fully formed spindle shows no distinction of central and mantle fibers, and no bodies which can be interpreted as centrospheres; all the fibers run from pole to pole.

The dissolution of the nuclear membrane is attended by a sudden narrowing of the spindle and a corresponding increase in length.

During the anaphase new (secondary) fibers, not to be confounded with mantle fibers, are put forth about the poles and meet in the equatorial region of the cell.

In the late anaphase the primary fibers, and soon after them the secondary fibers, begin to disintegrate, taking the appearance of beaded threads, and then of granules; at this time all of the stainable cytoplasm of the cell appears granular in texture.

The spindles of the second division at first have their axes parallel to the first cell plate. They are constructed out of the granular products arising from the disintegration of the first spindle.

The phenomena of the second spindles exactly repeat those of the first, except that four secondary spindles are formed by the union of the secondary fibers put forth during the anaphase.

The primary spindles become rotated about each other so as to bring the four daughter nuclei into the tetrahedral arrangement.

Cell plates are formed across the six spindles (two primary and four secondary), and in connection with them the separating walls of the spores are laid down.

Such a relation between the fibrillae of the kinoplasm and the cytoplasmic reticulum as Blackman (**5**) reports in Pinus, and Lawson (**8**) in *Cobaea scandens*, could not be verified. Between well developed spindle and cytoplasm are the three stages, (1) dotted fibers, (2) granules, (3) amorphous kinoplasm (structure too delicate for the microscope to reveal). The same phases in reverse order were traced in the first formation of the spindle.

This investigation was conducted in the Hull Botanical Laboratory of the University of Chicago during the spring and summer of 1899. The writer, while assuming full responsibility for the views expressed, takes pleasure in acknowledging his indebtedness to the members of the Botanical Staff for their courtesy and encouragement, and especially to Dr. Bradley M. Davis, under whose more immediate direction the work was undertaken.

*** Since the foregoing account was written (in February 1900), a comprehensive work on karyokinetic problems (**15a**) has been issued by Strasburger, in which, among other topics, he discusses the divisions of the spore mother cells of Osmunda, and the general formation of achromatic spindles in plants. He distinguishes two types of spindles, those possessing centrosomes and those without such controlling centers. The latter, which are characteristic of higher plants, are again subdivided into multipolar polyarch spindles, such as those of the spore mother cells of Equisetum, and multipolar diarch spindles, such as are common in various spermatophyte root-tips; and he seeks to minimize the divergence between these two extreme types of multipolar spindles by instancing numerous intergradations between them. My observations on Osmunda accord well with his view; for though I have described the spindle as bipolar from the beginning, its appearance soon after its inception is such that it may very properly be termed multipolar diarch. Strasburger recognizes in the spindle two kinds of fibers, which, having the same origin and reaction to stains, he prefers to call not central and mantle fibers, but supporting and attracting fibers (Stützfasern and Zugfasern). He pronounces against a power of movement in the chromosomes themselves, and attributes their withdrawal from the equatorial plate to the action of the attraction fibers. This conclusion is quite at variance with that expressed in the preceding pages. To the view that the nucleolus is used up to help complete the achromatic spindle, my observations, though not contradictory, are not altogether favorable; the spindle fibers are too

nearly complete before the disappearance of the nucleolus. In his renewed observations on Osmunda, Strasburger has noted the large and varying number (20–22) of chromatic groups in the spore mother cell. Each such group he regards as a chromosome pair. He finds the number of chromosomes in the prothallial cells is not so constantly twelve as he formerly (15) stated; it may reach sixteen or more. It is therefore certain that the current view as to the constancy of chromosome numbers cannot be maintained, at least as regards Osmunda.

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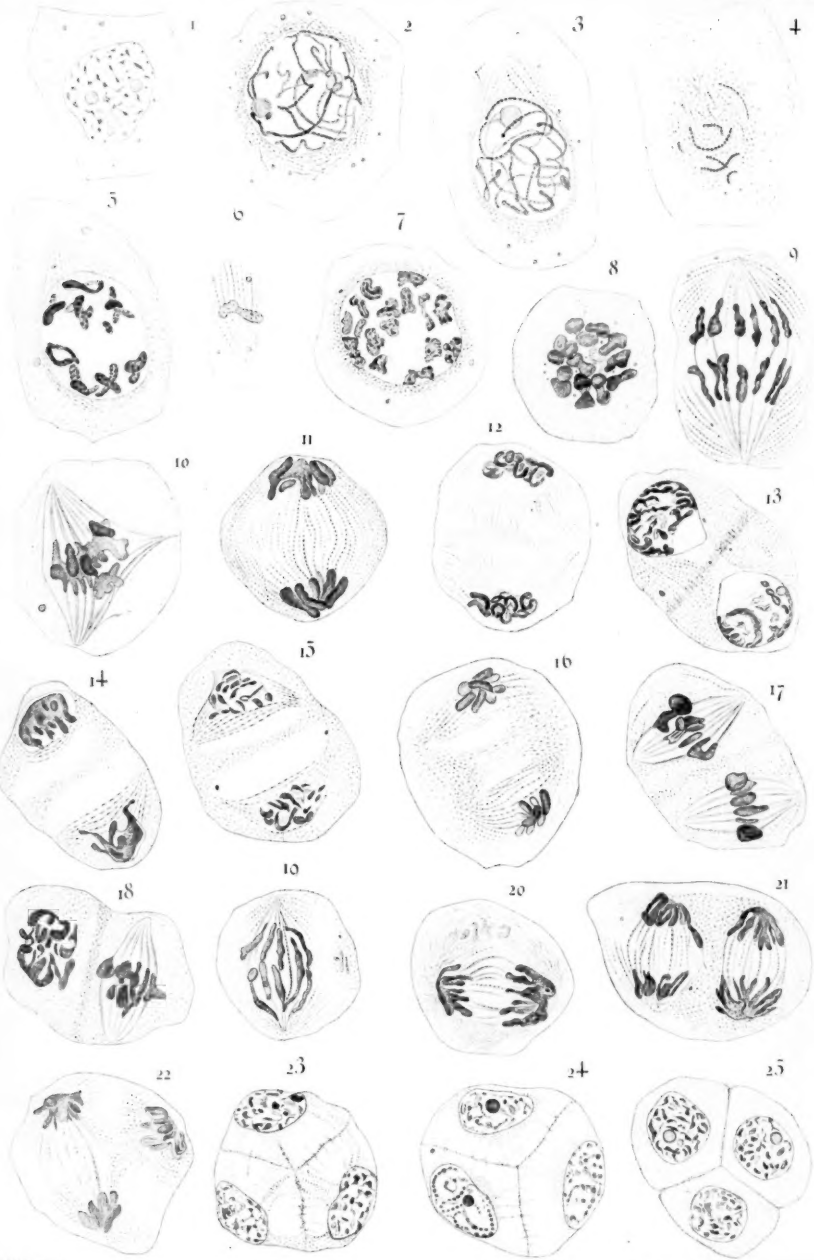

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EXPLANATION OF PLATE XXII.

The figures were outlined by the aid of an Abbé camera under the magnification given by a Zeiss apochromatic oil immersion lens 2^{mm} aper. 1.30, in combination with a Zeiss compensation ocular no. 8. In all cases the preparations were studied with the higher oculars, 12 and 18.

FIG. 1. Mother cell with resting nucleus.

FIG. 2. Mother cell with nucleus in spirem stage, and the kinoplasm distinctly granular and fibrillar.

FIG. 3. The same, somewhat more advanced, showing the bipolarity of the kinoplasmic mass.

FIG. 4. A section which cuts off only a portion of the nucleus.

FIG. 5. Chromosome stage.

FIG. 6. Portion of a cell, showing part of the equatorial region of the spindle.

FIG. 7. Later chromosome stage.

FIG. 8. Chromosomes in equatorial plate after the disappearance of the nuclear membrane.

FIG. 9. Anaphase, showing continuity of the primary fibers from pole to pole, and secondary fibers extending out from the poles.

FIG. 10. A tripolar spindle.

FIG. 11. Late anaphase.

FIG. 12. Late anaphase.

FIG. 13. Telophase; the primary spindle has quite disappeared.

FIGS. 14–16. Early stages of second division.

FIGS. 17–18. Metaphase of second division.

FIG. 19. Anaphase; secondary fibers beginning to form.

FIGS. 20–21. Late anaphase.

FIG. 22. Late anaphase; one of the primary spindles on the left side; the two other spindles shown have been formed out of the secondary fibers.

FIGS. 23–25. Successive telophases.

STUDIES ON CHROMOGENIC BACTERIA. I.

NOTES ON THE PIGMENT OF BACILLUS POLYCHROMOGENES.

E. M. CHAMOT and G. THIRY.

(WITH SIXTEEN FIGURES)

THE *Bacillus polychromogenes* was first isolated from a well water of Nancy by Macé[1] in 1894, and since its first discovery he has met with this same organism on five different occasions in well and conduit waters of that city. Two years after its discovery it was again isolated from the same well, and was found to possess the same characters as in the previous case. The organism was then described by one of us under the name of *Bacille polychrome*.[2]

These six colonies, found at different times, have varied neither in the original colonies nor on subsequent cultivation; varieties are, therefore, still unknown. Neither has it been possible to obtain variation by culture methods, for although one of the original colonies has been grown in the laboratory since 1894, part of the time in America, no change has been observed. It has also been impossible to obtain a non-chromogenic variety in spite of all attempts. It seems more than probable that this beautiful species will be met with by other investigators, and therefore, although the present article has to deal with the pigment, a few words regarding the characteristic features of the bacillus may not be out of place.

The *B. polychromogenes* was so named because of its peculiar power of giving a multiplicity of colors on ordinary culture media. On such media the organism produces at times blue, at

[1] MACÉ, E.: Traité pratique de Bactériologie. Ed. 3. 849–852. 1897. Atlas de Bactériologie, *pl 29*.

[2] THIRY, G.: Sur une bacterie produisant plusieures couleurs. C. R. de la Société de Biologie, 7 Nov. 1896.

Contribution a l'étude du polychromisme bactérien. Archives d. physiol. V. 9: 284–289. 1897.

others red, green, violet, purple, or yellow. These colors, as will be shown, can generally be controlled, thus permitting one to obtain at least part of the colors with certainty. This interesting fact has already been pointed out by the authors.[3] In addition to the pigment described below, colored insoluble microscopic crystals are also generally found on solid media. These crystalline aggregates are made up of irregular radiating clusters of fine needles of a deep blue color, and are probably not due to a crystallization of the pigment, but to crystals of some other substance stained by it. The composition of these crystals will be discussed in a future communication.

The organism liquefies gelatin, solidified blood serum, albumin (white of egg), fibrin, etc. In peptone solutions no indol is produced, neither does the bacillus produce gas in fermentation tubes filled with glucose or lactose peptone solutions at room temperature nor at incubator temperature.

The bacilli themselves are generally colorless, rarely they appear red or blue; in some of these latter cases the organism is uniformly stained, at others times only minute intra-bacillary granules are colored. The bacillus is polymorphic; not only does its form vary according to the nutritive media employed, but often there is great variation in form observed in different portions of the same medium. It has not been possible thus far to obtain with any degree of certainty a constant form on any culture medium, not even when employing one of definite composition (*i. e.*, a medium in which the source of nitrogen is not peptone etc., but a chemical compound of known composition such as asparagin or other bodies). In general the organism assumes the form of a short rod rounded at the ends, at other times it is spherical; again, long curved giant forms with swollen ends are seen. The bacilli are sometimes isolated, sometimes grouped (diplo-bacilli, chains). Coccus forms are likewise to be found in staphylo- and diplo- forms, or as tetrads and chains of eight cells.

[3] CHAMOT, E. et THIRY, G.: Bacille polychrome. Cultures et Spectre du Pigment. Communication à la Réunion Biologique de Nancy, Feb. 1898.

Usually the organism is motile, but the motion is always slow. Motility is to be seen in colored as well as in colorless individuals. It stains well by the ordinary methods and retains the stains by the method of Gram and that of Claudius. Greater morphological details would take us beyond the scope of the present note, namely, an account of some of the results of the study of the pigment produced by this bacillus on potatoes and on gelatin.

Growth on potatoes and the pigment formed.—On a medium as variable in composition as potatoes of different varieties and different ages of growth, it might be expected that there would be considerable variation in the colors of the pigment and nature of the growth of a chromogenic organism. The *B. polychromogenes* shows the effect of such changes in a most marked manner. Potatoes upon which this organism grows are colored variously yellow, greenish, red, violet, blue; the last color predominating but not constant. It was soon found, however, that a beautiful deep blue could be obtained, almost without fail, if the potatoes were first soaked in a dilute solution of sodium hydroxid (0.25 per cent. to 0.50 per cent.) containing a little calcium phosphate for twenty-four hours or less, depending upon the thickness of the pieces. Since this medium has served as the basis for the isolation of the pigment, and is being constantly employed by us in the study of the pigments of other chromogenes, it may not be out of place to describe our methods of preparation.

As large tubers as possible are chosen, such as are known to become mealy and porous on boiling. They are well washed in cold water, using a brush to aid in cleansing, and are dropped into boiling water with the skins on, and boiled till just cooked through. The water is then poured off, the potatoes allowed to cool somewhat, pealed, cut into slices 1 to 2^{cm} thick, and dropped into a dilute solution of sodium hydroxid, where they remain about eighteen hours. The supernatant liquid is then poured off, the slices drained and transferred to glass boxes 100^{mm} in diameter and 49 to 50^{mm} deep, with loosely fitting covers (in other words deep Petri dishes), a little water is added, and the

medium sterilized three days in succession in streaming steam.

When an active culture of *B. polychromogenes* is inoculated upon such a piece of potato, a deep blue very soluble pigment is produced, which diffuses slowly through the whole mass of the medium, coloring the latter an intense indigo blue. In from ten to fifteen days the coloring matter has penetrated the entire mass, and has colored it uniformly and so intensely that the slice of potato appears almost black. The more porous the potato, the more thoroughly and uniformly is it colored, since the pigment is reduced in the absence of air. For some time the color remains of the same intensity, then it becomes violet or purple, and then, owing either to the organism being no longer able to produce pigment to replace that being reduced by the various reducing substances present in the potato, or owing to the production of reducing agents by the culture itself, the color begins to fade. Decolorization proceeds more and more rapidly, the color at the surface of the medium exposed to the air being the last to disappear. The culture medium finally assumes a dirty brown color. Generally, cutting up the potato into thin slices and exposing them to the air leads to the production of a blue again by oxidation, providing the culture is not too old.

The blue pigment is very soluble in water, quite soluble in *dilute* alcohol, insoluble in strong alcohol, in ether, chloroform, benzine, etc. Water, therefore, is the best solvent; but unfortunately extraction with water removes such an amount of reducing substances that it was found necessary to employ dilute alcohol, the strength of the latter varying with the moisture present in the culture to be extracted.

In order to extract the pigment, the piece of culture medium is cut into thin slices and exposed to the air for a short time in order that as much of the blue pigment as possible shall be formed. Dilute alcohol is then poured over the material and allowed to act for some hours, the blue alcoholic solution is poured off, and a fresh addition of the solvent is made. This is repeated as long as any coloring matter can be removed. The

alcoholic solution of the pigment is then filtered through a carefully cleaned bacterial filter (Chamberland, Kitasato, d'Arsonval, or others). The first portions of the filtrate are rejected, owing to the fact that a chemical action takes place at first, doubtless owing to air present in the pores of the tubes; the result is the production of a beautiful purple-red liquid. After some 50^{cc}, more or less, have passed, the filtrate passes unchanged in color. It is then evaporated to a syrupy consistence at 50° to 60° C.; a higher temperature leads to reduction and decomposition of the pigment at this stage, owing to the presence of large amounts of sugars extracted from the potatoes. The thick deep-blue liquor is precipitated with strong alcohol (about 98 per cent.), the supernatant liquid poured off, and the precipitated pigment dissolved in a very small amount of distilled water and again reprecipitated with alcohol. This process is repeated as long as the alcohol seems to extract anything from the pigment. At this point the pigment is precipitated in such a finely divided condition that it refuses to settle completely, and cannot be retained on filter paper; a very small bacterial filter is therefore employed for the separations. The material is then carefully removed from the filter tube and dried. This dry amorphous powder has a grayish-blue color, and is completely soluble in water to a beautiful pure-blue color. Although we have reason to believe that it is still impure, the amount of impurity is doubtless so small that the pigment thus separated can be used as the basis of comparison, and for the reactions given in this paper. It cannot be made to crystallize, and is insoluble in all ordinary solvents, such as ether, petroleum ether, benzene, chloroform, amyl alcohol, etc.

If the blue aqueous solution is treated with a trace of acid, the blue is changed to a violet, a trifle more acid leads to the production of a beautiful purple (royal purple). An excess of acid gives rise to a red with more or less of a purple tint. So sensitive is the compound to acids that carbon dioxid causes a change of color. It was at first thought that when organic acids were employed, a color change resulted which was different

from that produced by inorganic acids. Later experiments seem to indicate that this is more probably due to a difference of intensity of action.

Ammonium hydroxid causes a change similar to that produced by acids, but the red color in this case is of a different tint from that obtained with the latter. The addition of acids to the purple-red ammoniacal solution restores the blue color, but if the acids are added in great excess the red tint of acid solutions results. It is worthy of note that a decided excess is necessary.

Fixed alkalies (potassium, sodium, barium hydroxids), in small amount, first produce a violet tint; if a little more of the reagent is added a pure blue results, but the color is somewhat paler than that of the original solution. When added in excess, the fixed alkalies give rise to a grass green solution. When the pigment has not been carefully purified, or when filtrates directly from a culture are employed, the change to green is much more rapid, and the amount of alkali required for its production is less. If the pigment is quite free from foreign bodies the green is rather persistent, but when impure rapidly fades away, leaving a yellowish liquid. The process of decolorization begins at the bottom and gradually extends upwards until the surface is reached; here, being in contact with the oxygen of the air, the color persists. If the yellowish alkaline liquid be shaken with air it immediately turns green, then blue; and if the agitation be continued there results a blue solution of almost the same intensity as the original. Allowed to stand undisturbed a reverse change is observed, namely, rapid decolorization passing through a green. The blue can be restored even after several days by shaking with air. The addition of alcohol to the yellowish solution produces a dirty yellow precipitate which turns blue the instant it comes in contact with oxygen. This phenomenon explains why it is that porous potatoes yield most pigment, and why cutting the colored medium into thin slices and exposing it to air before extracting, gives a larger quantity of the coloring matter; for it seems to be obvious that we have to do here with a case of oxidation.

Fixed alkalies added to the red solution resulting from the action of acids on the blue first restore the blue color, then, if in excess, produce a green, which in turn disappears as has just been described; and in like manner shaking with air or addition of hydrogen peroxid restores the blue. From neither acid nor from alkaline solution will solvents such as petroleum ether, ether, chloroform, benzene, amyl alcohol, etc., extract any coloring matter.

As to the important question whether the organism produces the blue pigment or a compound which turns blue in air, the writers do not yet feel justified in advancing an opinion.

When the clear blue solution (obtained by dissolving in water the coloring matter isolated from potatoes by the method described above) is placed before the spectroscope, a fairly well defined absorption band is seen in the neighborhood of the D line. The maximum intensity of this absorption band has approximately a wave-length of $\lambda = 594$, in the case of the purest pigment thus far obtained; its width and intensity varies, naturally, with the concentration and thickness of layer of the solution examined. There is also a slight darkening of the spectrum in the red, and a similar cutting off in the blue extending to the far violet. In the red this absorption seems to begin somewhere from $\lambda = 680$ to $\lambda = 690$, but is so gradual that no satisfactory measurements can be made. In the green, blue, and violet the increasing absorption is so gradual that no reliable decision can be made as to just where the absorption begins. *Fig. 1* gives the absorption spectrum of solutions 10^{mm} thick, containing 1^{gm} per liter of the purest pigment obtained; *fig. 2*, same solutions in layers 25^{mm} thick; *fig. 3*, same pigment in solutions of 2^{gm} per liter, examined in layers 10^{mm} thick. In 25^{mm} layers the absorption bands of these last solutions are too intense to permit of their being represented on the same scale as those figured. Blue solutions obtained by mere filtration of water extracts of colored potatoes usually show more marked absorption in the red and violet ends of the spectrum than do solutions of the pigment separated as previously described. *Fig. 16* shows the spectrum

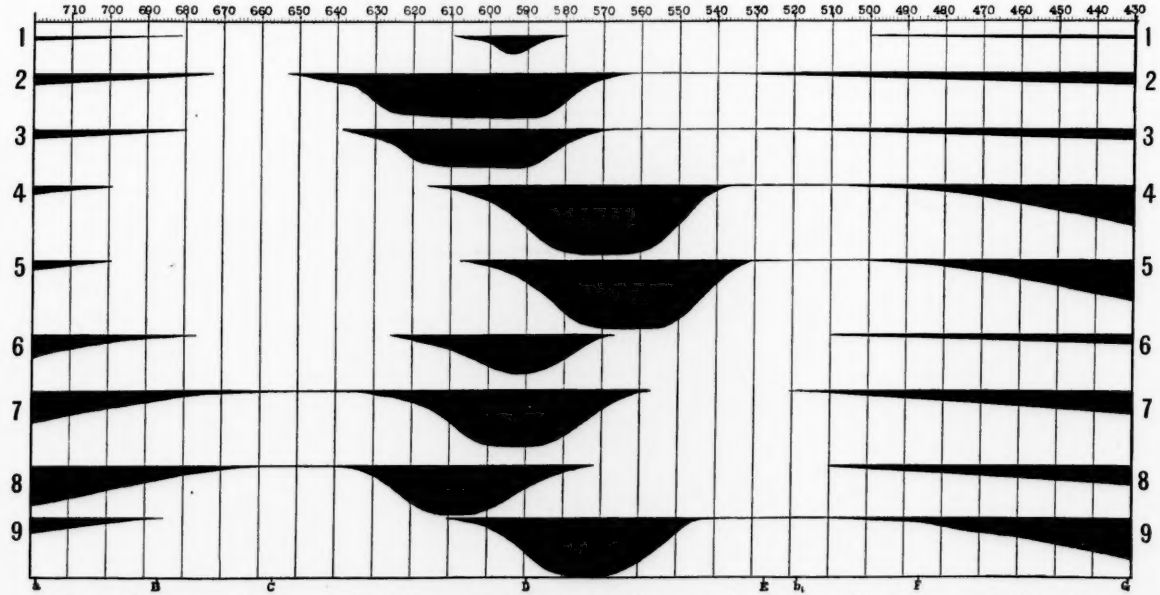

FIG. 1. One gram of pigment per liter in layer 10^{mm} thick.— FIG. 2. One gram of pigment per liter in layer 25^{mm} thick.— FIG. 3. Two grams pigment per liter in layer 10^{mm} thick.— FIG. 4. Two grams per liter, 10^{mm} layer, plus acetic acid.— FIG. 5. Two grams per liter, 10^{mm} layer, plus hydrochloric acid. — FIG. 6. Two grams per liter, 10^{mm} layer, plus ammonium hydroxid. — FIG. 7. Two grams per liter, 25^{mm} layer, plus sodium hydroxid until violet color results. — FIG. 8. Filtered aqueous extract of potato, plus sodium hydroxid 10^{mm} layer. Color intensity about equal to 2 grams pigment per liter. — FIG. 9. Same solution as that used for *fig. 8* after addition of acetic acid. 10^{mm} layer.

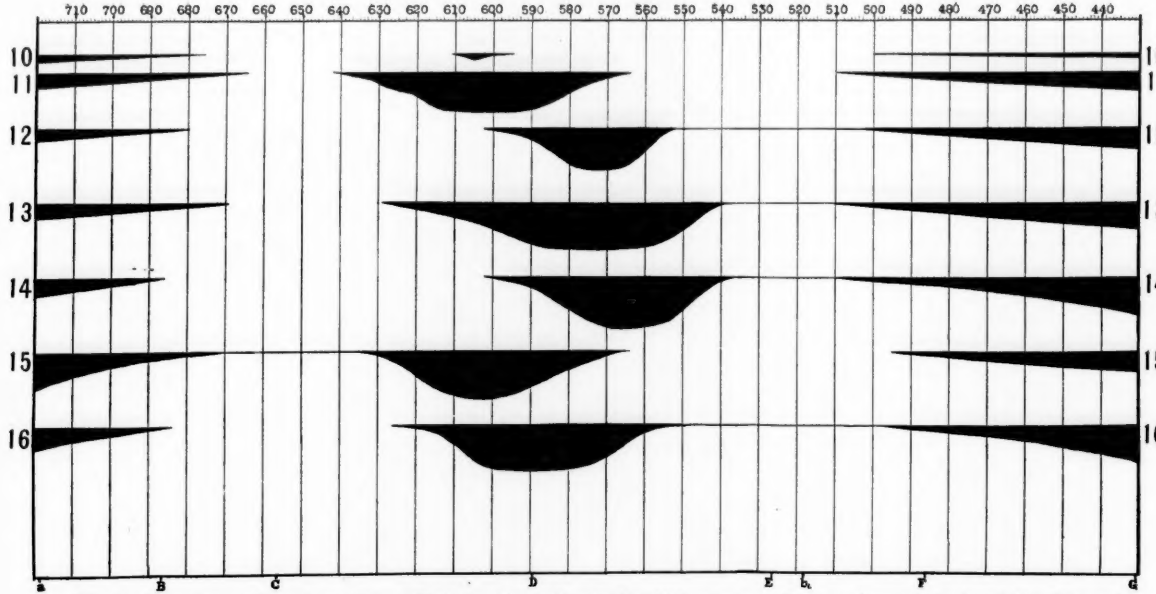

FIG. 10. Two grams pigment per liter, 25^{mm} layer, plus sodium hydroxid in excess. — FIG. 11. Green dichroic solution from gelatin culture three days old. — FIG. 12. Same solution as that used for *fig. 11* after addition of acetic acid. 10^{mm} layer as in *fig. 11*. — FIG. 13. Violet solution from growth on gelatin containing glucose. — FIG. 14. Same solution as that used for *fig. 13* after addition of acetic acid. — FIG. 15. Same solution as *fig. 13* after sodium hydroxid added in excess and mixture shaken with air until blue. — FIG. 16. Filtered aqueous extract from potatoes. 10^{mm} layer. Color intensity about equal to that of 2 grams pigment per liter.

obtained when 10^{mm} layers of such filtrates are examined, after having been diluted to a color intensity approximately equal to 2^{gm} per liter of purified pigment.

The addition of acids to the blue solutions causes an immediate displacement of the main band toward the violet, and increases its intensity at least twofold. The maximum intensity, of the main absorption band, of the now purple-red solutions is about $\lambda=570$. The absorption in the red seems to be diminished, but that in the blue and violet slightly increased. In *fig. 4*, the effect of acetic acid is shown; in *fig.* 5 that of hydrochloric acid. In each case solutions of 2^{gm} of the isolated pigment per liter, in layers 10^{mm} thick, were employed. *Fig. 9* shows the effect of acetic acid on the solutions used in *fig. 16*.

Ammonium hydroxid added in excess to the pure blue solution causes but little displacement of the main band, but greatly reduces its intensity, as also that of the bands in red and violet. This is not the result of dilution alone, however, since the addition of a corresponding volume of water gives no similar reduction in the intensities of the bands. *Fig. 6* shows the action of this reagent in slight excess on solutions of 2^{gm} of the pigment per liter, when examined in layers 10^{mm} in thickness.

Fixed alkalies when added in *very* small amount first produce a violet color. Such solutions yield an absorption spectrum shown in *fig. 7*, in which sodium hydroxid has been added to a 25^{mm} layer of a 2^{gm} per liter solution of pigment. Added in excess until a green results, the absorption band at D is almost completely destroyed, and the other bands about equally reduced in intensity. This effect is shown in *fig. 10*, where solid sodium hydroxid (to avoid dilution) has been added to solutions similar to those used for *fig. 7*. If the alkaline solution be shaken with air until it turns blue, a dark absorption band again appears, but not in the same position as in the original solutions, for it has been displaced toward the red end of the spectrum; its maximum intensity now falls between $\lambda=605$ and $\lambda=610$. This change in position is indicated in *fig. 6*, solutions similar to those used in *fig. 16* being employed.

Growth on gelatin.—Grown on gelatin of standard composition, liquefaction results and a green color is produced which gradually diffuses throughout the medium, the color being most intense near the surface. Generally after a few days a distinct red dichroism (fluorescence?) appears which increases with age.[4]

For the production of the coloring matter in large amount the following method is employed. A 10 per cent. solution of gelatin is made nutrient by adding 1 per cent. peptone (Witte or Chaputeau) and is rendered distinctly alkaline with sodium hydroxid. The medium is then clarified as usual with white of egg. It is then sterilized in the steam sterilizer in the ordinary manner. Gelatin of from 8 to 30 per cent. containing 1 to 5 per cent. peptone gives almost equally good results.

Since the coloring matter is formed only in the presence of an abundance of oxygen, cultures are made in large Fernbach flasks (antitoxin flasks) having a diameter of 20 to 25^{cm} at the bottom, the amount of gelatin added being sufficient to give a depth of about 1^{cm} (about 200^{cc}). The rapid production of as large quantities of pigment as possible being the end in view, the culture flasks thus prepared are inoculated with 1 to 2^{cc} of the liquefied gelatin poured from a very active culture of known purity, and are then placed in a closet protected from the light, where they are kept at a temperature of from 15° to 20°C. If the temperature rises much above 20° the production of pigment diminishes, and ceases if the culture be placed in the incubator. This is true for all culture media; the best results are obtained when the cultures are kept cool.

Fernbach flasks prepared as above begin to show at the end of 18 to 24 hours a decided green color which rapidly increases in intensity. At the end of three days the entire gelatin has assumed an intense, bright, grass-green, owing to the easy solubility of the pigment in water. Day by day the green becomes more intense and slowly darker, liquefaction also begins and with

[4] For diagnostic details the reader is referred to the articles already mentioned.

it a red dichroism makes its appearance; that is to say, the liquefied gelatin when viewed by reflected light is a pure grass-green; when viewed by transmitted light, red. The liquefied gelatin at this time cannot be distinguished in appearance from an alcoholic solution of chlorophyll. Like the latter it loses all its green tint by lamp, or ordinary gaslight (yellow light). That the coloring matter is, nevertheless, not chlorophyll will be seen from its absorption spectrum and from its behavior toward reagents.

The liquefaction of the gelatin starts at the center of the flask, where, owing to the slight convexity of the bottom, the layer of the medium is thinnest. The entire medium is soon completely liquefied, but still retains its intense green color. Gradually, however, the green changes to an olive tint and then fades away, as does also the dichroism, leaving a brownish-yellow turbid liquid.

In order to test the green coloring matter with reagents or to examine it spectroscopically, a perfectly clear solution must be obtained. This is effected by filtering the culture medium through one of the bacterial filters mentioned above. When liquefaction has not yet taken place, sufficient water is added to permit its passage through the filter tubes. When liquefaction is advanced, filtration is at once resorted to. The first runnings are rejected owing to changes in color, the result of chemical action in the pores of the filter.

The green non-dichroic solutions from young cultures give no absorption band in the neighborhood of the D line, or only the trace of one. The addition of a little acid produces a slight dichroism and a faint band appears. Acids added to any of the green dichroic solutions produce a red when in excess. Ammonium hydroxid gives also a somewhat similar color. Fixed alkalies destroy the dichroism and yield a fine clear green soon fading away. Shaking with air restores the color. These green alkaline solutions resemble the green ones obtained from young cultures and from potatoes in that they do not give an absorption band near D. The green pigment is insoluble in alcohol

and in the solvents enumerated above. The absorption spectra will be found represented in *figs. 10–16*.

If to the gelatin, prepared as has been described, a few grams of glucose, lactose, or similar bodies are added, instead of a green, a magnificent blue is obtained, permeating the culture medium completely and uniformly. The blue changes in a few days to a violet, then to a royal purple, and finally, as the culture grows old, the color disappears, the liquefied gelatin acquiring a brownish red color. Both the violet and the purple are dichroic, that is, are red by transmitted light. The liquefaction of the gelatin is considerably retarded by glucose, lactose, etc., as has been observed with many other species of bacteria.

The blue solution obtained by filtering young glucose-gelatin cultures reacts toward reagents in every way like the blue obtained from potatoes, that is, with acids first a violet, then a purple, and at last a red results; ammonia also produces a red; fixed alkalies give a green, soon fading and leaving a brownish-yellow solution which, when shaken with air, becomes first green, then blue. The absorption bands given by these solutions will be found in *figs. 10–16*.

Since it was found that these bands seemed to correspond in character, position, and intensity with those given by similarly colored or treated solutions of the pigment isolated from potatoes, no attempt has yet been made to isolate the coloring matter from gelatin. For the purposes of comparison, the filtered solutions were diluted with water until the colors given with reagents were approximately the same as those obtained with 2^{gm} per liter of the purified pigment.

The spectra of these gelatin solutions need but a few words in explanation. A few of the most important have been given in order that they may be compared with the similarly treated solutions shown by *figs. 1–9*.

Fig. 11 gives the absorption spectrum obtained with strongly dichroic green solutions. Acetic acid added to such solutions causes the change shown by *fig. 12*. *Fig. 13* represents the appearance seen when the violet solutions from cultures on

gelatin containing glucose, lactose, etc., are examined. It will be seen that the main band has suffered displacement toward the right, just as if an acid had acted slightly upon the coloring matter. Addition of acetic acid with the production of a purple-red color furnishes an absorption spectrum like that shown in *fig. 14* (compare this with *figs. 4, 5, 9*). *Fig. 15* is obtained when the violet solutions shown in *fig. 13* are treated with an excess of fixed alkali (in this case sodium hydroxid), and the mixture shaken with air until blue (compare with *fig. 8*).

The absorption curves represented on the plates are drawn to the scale of wave-lengths. The intensities are, of course, arbitrary, and are based upon a value which would permit of representing a spectrum such as that of *fig. 10*. Owing to the difficulty of judging the intensities of most of the solutions examined, particularly after long intervals of time, it is probable that the curves may not be perfectly accurate as to intensity. The positions of the bands, however, are correct within the limit of experimental error. The results given are averages from examinations of many cultures extending over a period of more than two years.

Most of the measurements have been made with a Krüss Universal Spektralapparat. It is, perhaps, needless to add that the usual precautions as to calibration, checking adjustments, etc., were observed.

Growth on agar-agar.—On nutrient agar prepared as usual and rendered slightly alkaline with sodium hydroxid, a more or less bluish-violet color is produced which diffuses through the upper part of the culture, but is reduced where the air cannot penetrate. In this case the reduction of the coloring matter has been proved by experiment to be largely due to the action of the culture medium, and not to products formed by the bacillus. In young cultures the bluish tint is the strongest, in old ones the red tint.

If to the nutrient agar glucose or lactose is added, the blue is very marked, but soon becomes violet, then purple. In the case of media inoculated by streak the change from blue to

violet starts near the inoculation streak and proceeds outward. This seems to give evidence of the formation by the organism of an acid or an acid acting substance. Such a hypothesis is supported by the fact that the red is not produced if calcium carbonate is suspended in the medium before it solidifies, thus furnishing a substance which will unite it with and neutralize any acid as soon as formed.

The comportment of the blue from agar toward reagents is identical with that of the blue from potatoes, and with that from gelatin containing glucose, etc. The absorption spectra of the different colored solutions from agar seem to correspond to those of like color obtained from other media, hence it has not been deemed necessary again to reproduce them.

Generalizations.—It will be noticed that potatoes, glucosed-gelatin, agar (especially if glucosed), etc., all yield a blue becoming violet, then more or less purple. Without taking more space to enumerate experiments, it can be stated that no case has yet been found where a blue has resulted in the absence of a sugar or similarly acting compound.

Agar-agar is probably closely related to the starches, and has been shown by Bauer[5] to contain a compound which is doubtless a sugar and, in all probability, to give rise to the formation of others through the action of acids or alkalies. Hence we have here the necessary substances to produce the blue pigment.

The violet colors on different media seem to be due to the action of an acid or acid acting substance. As the culture grows older there is more of this substance formed, and the violet changes to purple or to a red. This hypothesis is based upon the changes in position and character of the absorption bands; on the comportment of agar cultures containing calcium carbonate; and on the fact that the addition of acids to the blue solutions produce changes in color and in absorption spectra similar to those noticed in the aging of cultures. This acid may

[5] Journ. f. prak. Chem. II. **30**: 367. 1884. See also Lippmann, Chemie der Zuckerarten. Braunschweig. 1895.

possibly be acetic acid, since it has been possible to isolate alcohol and acetic acid from some cultures, yet this is by no means certain, since it is not clear whether these compounds existed preformed, or were the result of changes brought about by the analytical methods employed.

In the light of much experimental evidence extending over a considerable period of time, there seems to be no other hypothesis tenable than that all the different colors produced are simple derivatives of one and the same substance, that is to say, the organism does not produce different pigments on different media, as was first supposed, and as has been stated of many other chromogens.

The reactions and properties of this pigment do not correspond to those given by other investigators who have worked upon coloring matters of similar colors. The greens cannot be chlorophyll, nor can the blues be any one of the many cyanins which have been described. The pigment most closely resembles the coloring matters which have been isolated from lichens and from fungi, yet this resemblance is but slight.

The writers hope in a future communication to be able to announce something definite as to the nature of the pigment, and whether it belongs in reality, as now seems to be the case, to a new class of coloring matters heretofore unreported among the pigments of chromogenic bacteria.

One of the chief difficulties is to obtain sufficient purified pigment, for although its tinctorial power is very great there is but little of the material formed in each culture, and much of this is necessarily lost in the processes of purification. The problem becomes, therefore, in the first place, one of time, and cultivation of the organism on a large scale.

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NOTES ON THE DIVISION OF THE CELL AND NUCLEUS IN LIVERWORTS.

By J. M. Van Hook.

(WITH PLATE XXIII)

In the following pages are presented the results of some observations on the division of the cell and nucleus in the spore mother cell of Anthoceros, and in the vegetative cells of the gametophyte of Marchantia.

But little has been done in the cytology of the Hepaticae, aside from a study of the development of spores. Several papers were written by Farmer ('94–'95), whose detailed work has dealt, for the most part, with the cytology of reproductive cells. Along this same line Davis ('99) has worked out spore formation in Anthoceros, and his article upon "The spore mother cell of Anthoceros" strongly suggested the desirability of extending the study.

My observations on Anthoceros confirm his statement concerning the absence of centrospheres, but in regard to the disappearance of the connecting fibers and the formation of the cell plate by the thickening of strands of apparently undifferentiated cytoplasm which extended between the daughter nuclei my own results differ from his.

A cell plate seems to be formed in no wise different from that found in Marchantia, an account of which is given below, *i. e.*, the cell plate is laid down through the instrumentality of the connecting fibers (*figs. 11, 13*). Cytoplasmic strands similar to those figured by Davis are always found between the chloroplasts (*fig. 14*), but are undoubtedly *around* the spindle; and when a section includes only these strands, or if the connecting fibers be insufficiently stained, the appearance figured by him (his *fig. 25*) is presented. The same aspect is also displayed at a later stage, as in his *fig. 26*, but I am inclined to attribute

this to the persistence of the protoplasmic strands after the disappearance of the connecting fibers.

In order to see these plates as formed by the thickening of the spindle fibers, it was necessary to cut the sections two or three μ in thickness; for at this stage the daughter nuclei lie very close together, and the protoplasm about them is exceedingly dense. Staining was protracted two or three times the usual length of time, and the excess washed out with great care.

For my study there was placed at my disposal an abundance of excellent material of a species of Anthoceros collected and fixed by Professor Mottier near Naples in April 1898. On account of the large size of the cells and the greater clearness with which the finer details were brought out in the preparations, this material proved to be more favorable than that obtained from the Indiana species, in which many details were brought out with difficulty.

For the behavior of the centrosome in Marchantia we have only the abstract from Mottier ('98). He says: "In the vegetative cells of Marchantia I cannot assert with absolute certainty that a definite central body or centrosome exists in all cases, but I believe that such is the case. In some in which the kinoplasmic radiations are densely stained the dark center seems to be merely the point of union of the radiations, but if the stain be washed out so that the radiations are almost colorless, a well defined and densely stained central body is generally to be seen."

It is further stated that the centrospheres are attached to the nuclear membrane and are almost diametrically opposite by the time the chromosomes are differentiated or even earlier. Farmer's statement that the centrospheres exert a pulling strain upon the nucleus is substantiated. Frequently the nuclear membrane is drawn out by their influence into slender beaks.

For the study of karyokinesis in these vegetative cells the young and rapidly growing stalks of the archegoniophore of Marchantia was selected. These were fixed in the Flemming

solution as used by Mottier ('97). A number of stains were used, but the best general results were obtained by the triple method of aniline-safranin, gentian-violet, and orange G. The stains were varied both in kind and length of time of use, in accordance with the structures or parts of the cell which were to be brought out.

The cells from the more rapidly growing stalk of the archegoniophore are in general cubical, and a longitudinal section of the stalk will almost invariably result in meridional sections of the spindles. The resting nuclei are round and contain large conspicuous nucleoli. The nucleolus seems to lie in the cavity or vacuole. A careful examination reveals also a very delicate network of linin threads. These are clearly seen only in successful preparations when sufficiently magnified.

In the granular cytoplasm becoming vacuolate in older cells are situated many oval or rounded chloroplasts, together with metaplasmic inclusions which stain variously. The chloroplasts and metaplasmic inclusions generally collect about the poles of the nuclear spindle, thus obscuring the finer details and rendering observation difficult.

The first noticeable evidence of nuclear division is the pulling out of the nuclear membrane, generally in the direction of the long axis of the cell, into two points which are opposite each other and around which the chloroplasts tend to collect. A careful examination reveals a minute body at each point from which extend conspicuous radiations. These bodies with their radiations are the centrospheres. They seem undoubtedly to exert a great attractive force from the manner in which certain of the cell contents are drawn to them (*figs. 2, 4*). The way in which the nuclear membrane is often drawn out into very long points would seem to indicate further that the centrosphere exerts a pulling force upon it. The exact time of the appearance of the centrosome cannot be stated with certainty, because in the absence of the radiations it is impossible to distinguish the centrosome from other bodies appearing quite like it. From what is known of their behavior in other plants it is reasonable to

suppose that they appear before the radiations, for in fact centrosomes can be recognized during the reconstruction of the daughter nucleus after the radiations have disappeared (*fig. 11*). The dense center of the centrosphere, or the centrosome, is best seen previous to the appearance of the mature spindle as a dark round body at the center of many distinct radiations. There is no reason why it should not appear as a temporary body arising from some specialized cytoplasm, as kinoplasm.

As the nucleus increases in length, the radiations become exceedingly distinct and extend farther over the nuclear membrane (*fig. 2*). They also penetrate the nuclear membrane, for any section of the nucleus at this stage will show them in its interior. Before they meet at the middle from the two centrosomes to form the spindle, the linin threads have become more conspicuous with thickened or knotty portions appearing as chromatin (*fig. 3*). This forms a network filling the large central part of the nucleus, but does not seem to extend into the pointed ends. In no case was a continuous band or spirem observed, but a framework of linin at whose crossings or thickened parts chromatin gathers. These increase rapidly in size, separate by the dissolution of the intervening linin, and form a number of chromosomes. The number seems to vary. In one instance I counted five, in another eight. These are irregularly scattered within the nuclear cavity (*fig. 4*). They are often in the form of irregular masses, but sometimes in the shape of straight or bent rods varying in size (*fig. 5*). When arranged in the nuclear plate, however, they are generally U-shaped, and each is seen to be split longitudinally.

The spindle fibers have now extended to the region of the equator, the nuclear membrane disappears, and the spindle is formed. At its center the chromosomes gather, arranging themselves at the circumference of the equatorial plane. The spindle elongates very rapidly. It is pointed, with the centrospheres yet visible at either pole. In many cases it assumes a double curve like an elongated letter S (*fig. 8*). In almost every instance at this stage this curve may be observed. Even those that seem

straight may owe their appearance to the manner in which they have been sectioned; and such is undoubtedly the case from the position of the poles and the difficulty in making an exact meridional section. This shape seems to be due to the rapid lengthening of the spindle threads as soon as they have come together at the center. Spindles are often curved, or rather it happens that the poles do not have a common axis. This is observed in their formation, however, and before the centrosomes have taken diametrically opposed positions. The daughter segments separate and pass rapidly toward the poles, along the spindle fibers, which have now become straight. Here they crowd so closely together that they lose their individuality and appear as a broken mass of chromatin, which soon becomes enveloped in a new membrane forming the daughter nuclei (*fig. 9*).

The centrosomes become invisible or perhaps dissolve as soon as the polar radiations have disappeared. The connecting fibers now thicken in the equatorial region, the first indication of a cell plate (*figs. 9, 10*). This thickening begins at the center of the equatorial plane and gradually extends to its circumference, apparently pushing the fibers farther apart toward the cell membrane, until the spindle becomes very much bulged or rounded.

Concomitant with the bulging of the spindle, its connecting fibers begin to shorten and to dissolve at the poles (*fig. 11*). As they disappear toward the cell plate, which finally reaches the membrane, dividing the old cell completely, the daughter nuclei follow, and come to lie very near each other (*fig. 12*).

Thus the process of cell plate formation is almost identical with what I find in Anthoceros. It corresponds also with that described by Mottier ('97) for Lilium.

ANDERSON, INDIANA.

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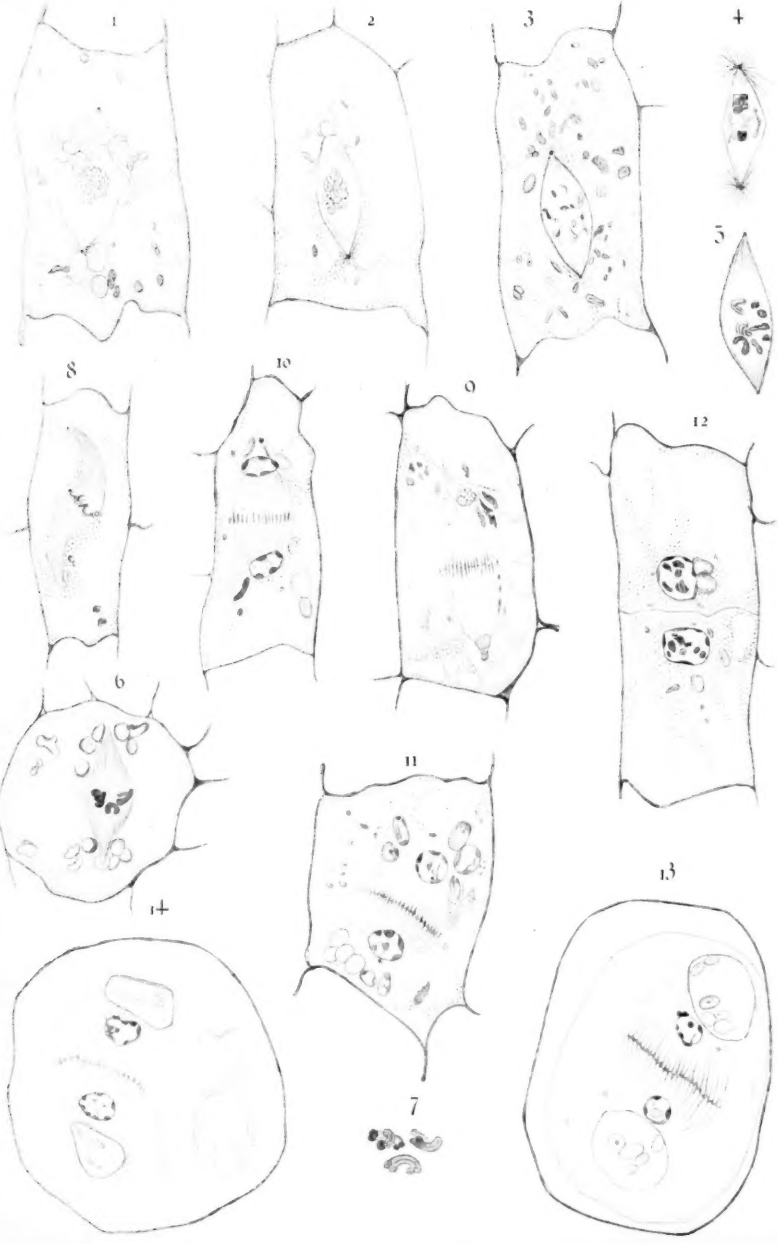

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EXPLANATION OF PLATE XXIII.

The figures were sketched with the aid of an Abbé camera lucida; *figs. 1, 2, 9, 10, 11, 12, 13, 14,* with a Bausch and Lomb $\frac{1}{12}$ oil immersion objective; the others with a Zeiss 2 mm oil immersion and compensating ocular 12.

All preparations were fixed in the Flemming solution used by Mottier ('97), and *figs. 11, 13, 14* were from sections 2 μ thick; the others, 5 μ. All were stained on the slide with Flemming's triple stain of safranin, gentian-violet, and orange G.

FIGS. 1–12, Marchantia; 13–14, Anthoceros.

FIG. 1. Nuclear figure from archegoniophore of Marchantia; the centrospheres are visible.

FIG. 2. The nuclear membrane is drawn out into points, and chloroplasts, with other bodies, are collected about the centrospheres.

FIG. 3. Nucleus previous to formation of the spindle; chromatin masses apparent in the linin net.

FIG. 4. Later stage in which the chromosomes are differentiated.

FIG. 5. Chromosomes collecting in equatorial region.

FIG. 6. Spindle stage with chromosomes arranged in equatorial plane; each chromosome is seen to be split longitudinally.

FIG. 7. Same, more highly magnified.

FIG. 8. A curved spindle.

FIG. 9. Formation of daughter nuclei.

FIG. 10. First appearance of nuclear plate.

FIG. 11. Bulged spindle, plate building toward the cell walls.

FIG. 12. Nuclear plate completed.

FIG. 13. Spore mother cell of Anthoceros, showing two of the nuclei only, and the nuclear plate in process of formation.

FIG. 14. Spore mother cell of Anthoceros, showing two of the nuclei with connecting fibers in which a cell plate is forming; cytoplasmic strands extend also between the chloroplasts and nuclei.

BRIEFER ARTICLES.

NEW SPECIES OF TRIMMATOSTROMA.

(WITH THREE TEXT FIGURES)

In the summer of 1898 I had my attention called to the pathological condition of the balsams in many parts of the province of Ontario. The disease, however, had not as yet done much damage, a dead branch

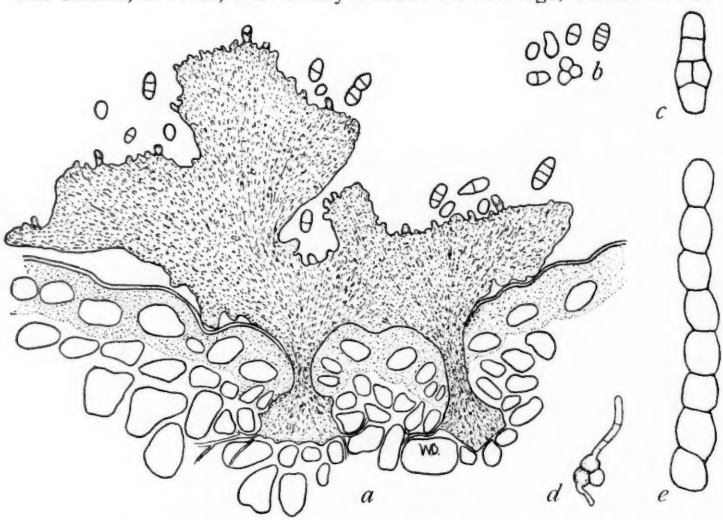

FIG. 1. *Trimmatostroma abietina: a*, cross section of a diseased leaf of *Abies balsamea*, showing the sporodochium (heavily shaded portion) and a number of loose conidia (×450); *b*, a group of conidia of various forms (×450); *c*, a single conidium (×1060); *d*, germinating conidium after thirty-six hours in hanging drop of water (×450); *e*, chain of unicellular conidia (×1060).

here and there being the only warning sign of what later proved itself to be a disastrous outbreak. The woods at that time presented a peculiar mottled appearance. It was thought at the time that in this case we had to deal with a fungus form of some kind, but all efforts to collect material having it in fructification proved futile. Sections of

the diseased leaves showed the presence of a much branched mycelium made up of hyphæ which were both intercellular and intracellular. Owing to an interruption of several months, caused by the pressure of classroom work and laboratory duties, no further observations were made until the following summer (1899). The progress of destruction during this interval was far beyond expectation. Trees which but nine months before showed only a dead branch or two had now entirely succumbed, and the disease was spreading rapidly, many new points of infection being noticed. Any doubts as to the full malignancy of the disease which may have existed when it first made its appearance were now dispelled, and it was but too evident that the injury to the balsam forests would be great. The diseased leaves at this time showed the presence of numerous black warty tubercles which proved to be the fruiting masses (sporodochia). A quantity of material was collected and brought to the laboratory for further examination and study. Pure cultures were made on many different media in tubes and plates, and great difficulty was experienced in obtaining a normal development and in many cases any growth at all. Blood serum (Loeffler's mixture for diphtheria cultures, no. 8), potato agar, potato, beef agar (acid, neutral, and alkaline), and nutrient gelatin, were all tried and found serviceable as indicated in the order mentioned. Hanging drop cultures were made from the plate cultures which proved pure. In many cases the spores refused to germinate in water. Those which germinated at all did so within thirty-six hours and the majority within twenty-four, a single germ tube being put out from each spore, or in some of the multicellular forms one from each cell (*fig. 1, d*). From observations which I have made upon cultures and sections of imbedded material, I would characterize the form as follows:

FIG. 2. A culture tube showing the growth of *Trimmatostroma abietina* upon blood serum.

Trimmatostroma abietina, n. sp.— Mycelium perennial, intercellular or intracellular, hibernating during the first winter in the tissues of

the host, the following spring giving rise to conidiophores which issue from the tissues of the leaves and branches in dense fascicles forming a diffuse sporodochium. The conidiophores nearly hyaline or tinged olive brown, 4.5μ × 20–30μ, septate, sparsely branched, bearing the

FIG. 3. Forest scene in Ontario, near Guelph, showing the appearance of a diseased area.

conidia terminally. Conidia in chains of many different kinds, all of a dark brown olivaceous color, with epispore slightly roughened, the majority oblong or spherical, usually straight, a few inequilateral; some continuous, spherical, 5μ in diameter; others septate, 2 to

5-celled, 5 to 6μ×8–16μ, not constricted at the septa; and a few of the muriform type, 5μ×10μ.

It will readily be seen from these characters that the form belongs to the Hyphomycetes, and in this group to the "series" Tuberculariæ dermatieæ, "section" Phragmosporae. Further than this its identification presents many difficulties. While a large majority of the conidia are horizontally septate, and hence must be classed under Phragmosporae, a small number have a longitudinal septum, but owing to the fact that they are borne in chains the fungus must belong to the genus Trimmatostroma. A careful examination of the literature of the subject fails to show any reference to anything of a similar form upon any coniferous tree.

Although, so far, the only hosts upon which this species has been found are *Abies alba* and *Abies balsamea*, it is very probable that the spruces (Picea) are not immune. Further search will be made in the hope of discovering a more mature stage of this form.

I wish to acknowledge my indebtedness to Mr. B. T. Galloway and Miss Patterson, of Washington, D. C., for having kindly assisted me in the examination of material and reference literature.— M. W. DOHERTY, *Agricultural College, Guelph, Ontario.*

THE INTERNATIONAL BOTANICAL CONGRESS.

[Through the courtesy of our associate, Professor L. Guignard, we have received from the general secretary, M. E. Perrot, the following account of the recent meeting in Paris of the International Botanical Congress, whose sessions were held during the first ten days of October. The president of the Congress was M. le Dr. de Seynes, a former president of the Botanical Society of France; while the different sessions were presided over by MM. Drake del Castillo, Dutailly, Flahault, Mussat, and Rouy. Among the foreigners present, we note the names of Borzi, Burnat, Britton (N. L.), Chodat, Czapek, Dyer (Th.), Errera, Filarsky, Gallardo, Gamble, Greshoff, Istvanffi, Koltz, Magnus, Maiden, Micheli, Niederlein, Pfitzer, and others.—EDS.]

1. SUBJECTS INTRODUCED FOR DISCUSSION BY VARIOUS MEMBERS.

Methods of facilitating popular instruction concerning mushrooms.— The introductory address was given by M. Rolland, who was followed by several members in the presentation of opinions. It was voted finally that instruction in mycology should be given down to the primary school, beginning with the recognition of poisonous species, and especially species of Amanita and Volvaria, the eating of which is

almost always followed by death. It was further stated that the illustrations of mushrooms intended for instruction should always be made under the direction of professional mycologists.

Unification of methods employed in the determination of molds and yeasts.—To make comparable the diagnoses of these organisms, MM. Lutz and Guéguen presented a report which proposed that investigators should substitute artificial culture media of definite and constant composition for natural culture media.

Adoption of an international unit of micrometric measurement.—After an address by M. Mussat, the Congress urged all botanists to use the micrometric μ.

Periodicity of international congresses.—It was decided that hereafter international congresses should be held every five years and at different places. The Congress of 1905 was appointed for Vienna, with Professors Wiesner and von Wettstein in charge of the organization.

Nomenclature.—The Congress declared itself incompetent to revise the laws of nomenclature, but it arranged for conference upon the subject among the principal botanical societies and establishments of different countries. A representative committee will be appointed to elaborate a plan to be discussed at the Congress of 1905. M. John Briquet, curator of the Delessert Herbarium, was asked to serve as a channel of communication among taxonomists interested in the solution of the question of nomenclature, and to centralize all the correspondence.

Phytogeographic nomenclature.—In view of the rapid extension of phytogeography, and at the suggestion of M. Flahault, the Congress voted that all "geobotanists" should be urged to come to an agreement in reference to the terminology of the general facts of phytogeography; and to establish in the principal languages the precise synonymy of the terms recommended for use by travelers and geographers.

Establishment of an international periodical for the publication of new botanical names.—The subject was presented by M. Hua, who showed the advantages of some authoritative list of new names, both in avoiding the multiplication of synonyms and in simplifying publication. The Congress authorized M. Hua to devise a plan for realizing this very difficult project.

II. THE PRINCIPAL SCIENTIFIC PAPERS.

About thirty original contributions were presented by their authors, and gave rise to more or less discussion. The papers were as follows: M. BONDIER on the influence of the soil and vegetation on the development of mushrooms; M. DANGEARD on the present status of knowledge concerning reproduction among the higher mushrooms; M. R. MAIRE on the behavior of the nucleus among mushrooms; DR. C. B. PLOWRIGHT and PROFESSOR MAGNUS on the biology of the Uredineæ; MM. CHODAT and RADAIS on the pure culture of algæ; M. CHODAT on the reactions of the nucleus to parasitism and symbiosis.

The flora of central Africa was discussed by MM. DE WILDEMAN, HUA, and A. CHEVALIER, and contrasted with that of the Amazon region, as described by M. HUBER. M. E. J. CAMUS discussed the flora of Morocco, and Dr. N. L. BRITTON gave new and interesting facts concerning the flora of the Klondike.

The physiological and anatomical papers were by MM. HOCHREUTINER, GERBER, and GIDON; while M. MARTEL presented studies on Cruciflorae, M. BEILLE on Disciflorae, M. DUTAILLY on Geum, and M. CLOS on bracts, sepals, petals, stipules, etc.

M. ANGEL GALLARDO spoke highly of the employment of the statistical method in the study of variation; while M. HUGO DE VRIES spoke upon the general subject of variation in the plant kingdom. M. PH. DE VILMORIN described a curious case of selection in *Anthriscus sylvestris;* MM. LÉVEILLE and HY spoke of hybrids; M. KRASAN treated of varieties, races, and forms; and M. GILLOT pointed out modifications which appear in local floras.

A discussion concerning the exchange of material among herbaria was participated in by MM. MOULLEFARIN, DRAKE DEL CASTILLO, and FLAHAULT.

The Congress varied the scientific conferences with visits to several collections and plantations.

NOTES ON THE FLORA OF THE BANKS AND SOUNDS AT BEAUFORT, N. C.

THE coast of North Carolina near Beaufort, like most of our Atlantic coast from Long Island southward, is bordered by a line of narrow, sandy islands, or "banks," separated from the mainland by shallow sounds from one to five miles wide. These banks vary in

width from a quarter of a mile to a mile and a half, and in height from nearly sea level, in the case of certain salt marshes, to perhaps thirty feet in the case of some of the highest of the wind-blown dunes. The conditions prevailing on the higher parts of these banks are quite peculiar, for though the shifting sandy soil holds very little water, the air is very damp, since during the growing season the prevailing wind is a strong sea breeze saturated with moisture from the warm water of the Gulf Stream. Thus is to be accounted for, perhaps, the frequent occurrence here of many plants not usually found in so dry a soil, and the striking abundance of epiphytic lichens, liverworts, mosses, and ferns.

In the following pages are noted some of the interesting features of the flora of Bogue Bank and Shackleford Bank, observed during a three weeks' stay at the U. S. Fish Commission Laboratory at Beaufort, in June 1899.

The effect of physiographic agencies on the two banks above mentioned is at the present time quite different. Bogue Bank, at the point studied, seems to have reached a stage of development where it is comparatively stable. It has an outer border, or sea wall as it were, of high dunes covered with *Andropogon maritimus*, and firmly bound by its long tough rhizomes into barriers very resistant to the destructive advances of both the sea and the wind. On these dunes, growing with the Andropogon and within easy reach of the spray, are found scattered scrubby specimens of *Juniperus Virginiana* and large patches of *Ilex Cassine*, both overrun with *Ampelopsis quinquefolia*, *Rhus Toxicodendron*, *Smilax Bona-nox*, and *Melothria pendula*. Scattered among them are found *Chenopodium Botrys*, *Diodia teres*, *Œnothera humifusa*, and *Solidago sempervirens*.

As we go inward and downward from the outer crest of the dunes the forms mentioned give way to, or become mixed with, a very large number of other forms, making a flora much like that immediately to be described for Shackleford Bank.

On the latter bank, instead of the outer border of comparatively stable dunes, we find a shifting waste of wind-blown sand, stretching in a quarter of a mile or more from the water's edge, and reaching its greatest height at its inner border, where it is encroaching upon and rapidly burying what remains of the forest that once covered most of the bank.

For a hundred yards in from the water the only plants seen are the

everpresent *Euphorbia polygonifolia* and small tufts of Andropogon. At the inner edge of this shifting sand plain its surface is often ten or twelve feet above the old forest floor upon which it is advancing. It ends abruptly in a steep slope down which the sand rolls after being blown along the surface of the plain.

From the foot of this slope to the inner or sound side of the bank, a distance of nearly a mile, the surface is rolling and well covered by a thick scrubby forest made up chiefly of *Quercus virens*, *Q. aquatica*, and *Q. nigra*, with *Ilex opaca*, *Morus rubra*, *Persea Carolinensis*, *Carpinus Caroliniana*, *Juniperus Virginiana*, and *Pinus Taeda*. The highest of these trees near the middle of the bank reach a height of twenty-five or thirty feet, some of the pines perhaps exceeding this limit in the wider parts of the island.

The shrubby undergrowth between these trees is made up chiefly of *Myrica Gale*, *Ilex glabra*, *Ilex Cassine*. The latter is the most abundant of all, and near the outer border of the forest often nearly equals the trees in height. The number of species in the undergrowth becomes larger as we go in from the advancing sand plain, and soon includes many herbs, the covering of vegetation being very dense from the middle of the bank inward.

The distribution of species within this area is dependent largely upon the level of the surface. Pinus, Juniperus, and Morus occur chiefly upon the elevations, while *Ilex opaca*, Carpinus, and Persea, with the three species of Quercus noted above, occupy the hollows. These trees and the shrubby undergrowth are everywhere overgrown with dense tangles of *Berchemia volubilis*, *Vitis rotundifolia*, *Rhus Toxicodendron*, *Ampelopsis quinquefolia*, *Smilax Bona-nox*, *S. tamnoides*, and *S. rotundifolia;* often also with *Cissus stans*, *Gonolobus tuberosus*, and *Melothria pendula*.

Where the vegetation is densest, the trunks and branches of the trees and shrubs are covered with epiphytes. *Polypodium incanum* forms straggling clumps, usually on *Quercus virens*, but occasionally on the other oaks or even on the pine, becoming well established only when the tree is old enough to have a pretty rough bark. Species of Frullania, Liochlaena, Lejeunia, and Archilejeunia, form reddish or yellowish patches on the trunks of the trees, or in the case of one or two species, the delicate branches creep at the very bottoms of the deep grooves in the rough bark. Lichens, in great variety for so restricted an area, cover every available surface. On the oaks, pines, and

mulberries occur Parmelia, Ramalina, Usnea, and other fruticose and foliaceous forms, while the smooth barked hollies, Persea, and Myrica are literally covered from the trunk out to branches of only two or three years' growth with crustaceous Placodiums, Buellias, Lecidias, and others, in great variety of form and outline. One of the most striking species, occurring chiefly on the holly, forms brilliant blood red patches as large as the hand. A trunk blotched with this, intermingled with others of various shades of yellow, green, brown, and black, each patch with its clear, black outline, and often dotted with fruits of a different color, is a veritable mosaic of color.

In the lowest portions, near the middle of the banks, occur small pools of dirty water, bordered with sedges and grasses, and often also with stemless palmettos (*Sabal Adansonia*). In other, often well shaded, hollows dense patches of *Saururus cernuus* are found, and still other more open ones are completely covered with the gigantic *Sagittaria lancifolia*. Finally, among the shrubs and other herbs of these hollows are found three of the five pteridophytes seen on the banks. Those occurring here are *Aspidium Thelypteris*, *Onoclea sensibilis*, and *Osmunda regalis*. Of the other two, *Asplenium ebeneum* occurs on the drier shaded portions, and *Polypodium incanum*, as noted above, is an epiphyte.

At the inner edge of the encroaching sand plain the process of burying the old surface of the bank can be seen in all stages of progress. Just in from the foot of the slope, that is, on the outer border of the forest, many of the trees are seen to be dead, though the sand has not yet touched them. The junipers and yaupons, however, persist, and even when buried to the waist in the slope of drifting sand still look fresh and green at the top. As one goes seaward from this border between the shifting sand and the forest, these few green tops finally disappear, and then for one hundred and fifty yards or more one finds only the dead tops of trees of which all but the upper third, that is about six or eight feet, have been buried by the sand. Of these tree tops those nearest the existing forest are mostly denuded of bark, and then a little farther out one sees that the soft sapwood has been entirely carved away by the furious blasts of sand. Yet in many cases, a hundred feet from the edge of the sand slope, they are still capped by dense mats of Berchemia, Ampelopsis, or Rhus, and on the leeward side of the branches many lichens still flourish.

In the comparatively few days spent in examining these banks it

became evident that many ecological problems are here presented for solution. Interesting results ought certainly to be gained from a comparison of the distribution and anatomical structure of the forms occurring here with those of the same or similar forms on others of these banks farther north, where the atmospheric moisture conditions must, it would seem, be quite different.

In all nearly a hundred vascular plants were collected and identified, chiefly by Mr. W. C. Coker.

NOTE ON THE ALGÆ OF BOGUE AND CORE SOUNDS.

The nature of the bottom of the ocean and sounds near Beaufort is such as to provide few habitats of the sort most frequented by marine algae. There are no rocks whatever, except one or two stone wharves near the town, and several short breakwaters near the inlet from the ocean. Rock pebbles even are practically wanting, any as large as a pea being seldom found on the natural beaches. It will not be surprising, therefore, to find that the algal flora is comparatively poor in species, and is made up in numbers chiefly of those forms which attach themselves to shells lying on the sandy or muddy bottom.

Probably the most abundant alga in the sounds, except possibly *Ulva lactuca*, is *Hypnea musciformis*, which grows in thick snarled tufts on every available substratum. Scattered quite generally among the latter are tufts of *Dictyota dichotoma*, here near its northern limit, together with abundant specimens of *Rhabdonia tenera* and *Gracillaria confervoides*.

Codium tomentosum sometimes also occurs on shells in the sounds, but is much more frequent on the rocks of the breakwaters. On these same rocks we find Dictyota in abundance, often accompanied by the related form *Padina pavonia*. More abundant than all other algæ here, however, is *Sargassum vulgare*, which covers the inner breakwaters with thick tufts half a yard in length. On the Sargassum and the much less frequent Fucus, *Ectocarpus siliqulosus* and other Ectocarpus species are very abundant. Among the coarser forms, especially on the more exposed parts of the breakwater, grow Enteromorpha and most of the more delicate Rhodophyceæ that are found near Beaufort. Chief among the latter are *Dasya elegans*, *Erythrotrichia ceramicola*, *Gelidium crinale*, *Grinnellia Americana*, and *Trentepohlia virgatula*, with several species each of Callithamnion, Ceramium, and Polysiphonia.

On the leaves of *Zostera marina*, which abounds throughout the sounds *Melobesia pustulata* and probably other species are very common.

Altogether between twenty-five and thirty species were found, the most interesting feature of the flora being perhaps the occurrence here, at what must be nearly their northern limit, of the tropical or subtropical forms Codium, Dictyota, and Padina.—DUNCAN S. JOHNSON, *Johns Hopkins University*.

NOTES ON THE VALIDITY OF ASPLENIUM EBENOIDES AS A SPECIES.[1]

A RECENT visit, in company with Mr. Charles L. Pollard, to the somewhat famous locality for this fern at Havana, Hale county, Alabama, has led me to review what has been written upon the question of its hybridity, and to offer here a few comments, both upon its occurrence in Alabama and regarding its status as a species.

The theory of the hybridity of *Asplenium ebenoides* originated with Berkeley,[2] when, in publishing Scott's manuscript name in 1866, he expressed an opinion that the fern might well be a hybrid between *Asplenium ebeneum* and *Camptosorus rhizophyllus*. No considerable amount has since been written, but, considering the scattering references available, I think it may fairly be said that the weight of authority has been in support of Berkeley's proposition. Professor Coulter, in mentioning the discovery (1882) of new stations for this "suspicious species," remarked[3] that the "burden of testimony all seems to be in favor of that idea." Both Mr. Redfield[4] and Professor Eaton[5] regarded the fern as a probable hybrid; while the most concise favorable comment is that of Mr. George E. Davenport,[6] who cites it as "probably the best example of a fern hybrid that we have, the infrequency of its occurrence, the presence always of *Camptosorus* and *Asplenium ebeneum*, and the few plants found in the recorded stations, all going to favor the hypothesis of hybridization." In 1896 Professor Underwood, having visited the Havana locality, made the statements,[7]

[1] Published by permission of the Secretary of the Smithsonian Institution.

[2] Journ. Roy. Hort. Soc. 87. *pl. 2, fig. 1*. 1866.

[3] BOT. GAZ. **7**: 37. 1882.

[4] Proc. Acad. Nat. Sci., Phila. Dec. 1874.

[5] Ferns of N. Am. **1**: 26.

[6] BOT. GAZ. **19**: 492 *et seq.* 1894.

[7] BOT. GAZ. **22**: 410. 1896.

that "the display of the species at Havana clearly demonstrates that it is not a hybrid at all," that the fern appeared "to be multiplying, as many young plants were seen in the crevices," and that "this myth of hybridity may be put aside, for *Asplenium ebenoides* is as clearly defined a species as we possess in the genus Asplenium, and has no near relatives outside of its own genus." It seems to me that the conclusion adopted by Professor Underwood does not follow necessarily from the facts observed, and is perhaps less logical than the assumption, on the other hand, that the fern is a hybrid because always found under what may be termed "suspicious circumstances."

In the particularly rugged conglomerate gorge where *A. ebenoides* grows at Havana, it is indeed abundant; plants in all stages of growth abound, from the prothallus to the independent small plant and those ranging to the maximum of ten or eleven inches. *Asplenium platyneuron* (*A. ebeneum*) is common in the near vicinity, but Camptosorus is not in great evidence, in fact, was not seen either by Mr. Pollard or myself (who made no especial search for it), though it had previously been found here in small quantity. Now, if it be agreed, as Professor Underwood assumes, that a test of this fern's hybridity consists in its inability to reproduce through its spores, most assuredly *A. ebenoides* may not be classed as a hybrid; for the presence of prothalli and young plants in such numbers precludes the possibility of their having arisen in any other way, such as by a wholesale crossing of the suspected parents. The chances of frequent crossing are indeed small, since the Camptosorus is present in such limited quantity; while the groups of young plants are too far distant from mature ones to suppose that they arose from proliferous buds; besides the prothalli would arise only from spores.

The question naturally arises, then, as to whether the fact of the fern's fertility effectually disposes of the supposition of its hybridity. May it not be a *fertile* hybrid? The only really well-authenticated instances of hybridization between species, of which I am aware, are those of *Phyllitis scolopendrium* (*Scolopendrium vulgare*) with *Ceterach officinarum*, *Polypodium vulgare elegantissimum* with *Phlebodium aureum*, and *Polystichum aculeatum* with *Polystichum angulare*. In the first case[8] the cross was effected by Lowe. Three fronds were sent by him to Mr. C. T. Druery, who has explained carefully[9] their intermediate

[8] DRUERY: Gardener's Chronicle Sept. 1895.

[9] Journ. Roy. Hort. Soc. **24**: 292. 1900.

character, and in a recent letter briefly describes them as "scaleless fronds of true Ceterach form, but somewhat confluent at the tips, and with faced sori in pairs on the lower pinnae, but with single asplenioid sori on the upper portions." In the second case[10] the evidence is no less convincing, and the hybridity of the fern may be regarded as proven beyond question by anatomical studies, and the fact that the cross was several times repeated. In the third case[11] the cross (accomplished by Lowe) was between a cristate *angulare* and a dense form of *aculeatum*, resulting in a cruciate *aculeatum*. As to the fertility of these three hybrids: the sporangia of the first, contained in the fronds sent to Mr. Druery, were immature, so that no test of fertility could be made.[12] In the second case the spores have so far proven incapable of germination; but this sterility, Farmer suggests,[13] may be due to the circumstance of excessive vegetative development, for in the plainer fronds, reverting somewhat to the *vulgare* type, the spores are much better developed. In the third instance, I am informed by Mr. Druery, that, though producing spores freely, the plant proved barren in Mr. Lowe's hands, yet by others it has been found fertile, and a few plants have been raised which are freely fertile, and have given rise to a number of fine plants now in existence. Undoubtedly the parents of the latter hybrid are closely related, and a greater fertility might upon this account be expected; but had it proven infertile, as with the Polypodium-Phlebodium cross, it seems that even these two negative results might not properly be taken as criteria to justify the assumption that fern hybrids are sterile as a matter of course. To my mind the supposition of hybridity for *A. ebenoides* is not weakened by the discovery of its evident fertility. I am told by Mr. H. J. Webber that in the case of spermatophyte hybrids between distinct species the percentage of fertility is very much in excess of the common belief; the hundreds of sterile crosses here count for nought as an argument leading us to *expect* sterility, in the presence of an equal host of fertile ones. And so it may be in the case of fern hybrids. It appears entirely gratuitous to assume that there can be

[10] FARMER: Annals of Botany **11**: 533 *et seq.* 1897.

[11] LOWE, Fifty years fern growing. 1896. DRUERY: Journ. Roy. Hort. Soc. **24**: 292. 1900.

[12] Mr. Druery states that these fronds are still in his possession, but that the plant has probably not survived.

[13] FARMER: Annals of Botany **11**: 533 *et seq.* 1897.

no such thing as fertile hybrids between fern species. If we grant the specific distinctness of *aculeatum* and *angulare*, the converse has now been proven. The fertility of certain varietal crosses seems well established, and it may be regarded as by no means improbable that, with the use of careful methods, the same may be unquestionably proven in the case of a good number of specific hybrids.

Regarding the relationship of *A. ebenoides* to its supposed parents, Professor Eaton remarked[14] that "while it differs from the first [Camptosorus] by its dark and shining stalk and rachis, in its free veins, and by its pinnatifid or sub-pinnate frond, it resembles it strongly in the prolonged and slender apex, and especially in the proliferous buds; and in the very respects in which it differs from this it resembles the other." These very features of resemblance appealed to Berkeley with sufficient force to induce him to advance the hybridity proposition; but Professor Underwood apparently attaches little importance to them, considering the plant closely allied to *Asplenium pinnatifidum*, in which he is supported by Professor Murrill.[15] The fern seems to me to exhibit conspicuously certain characters of both its supposed parents, not the least important of which is the more or less frequent anastomosing of the veinlets. This fact is easily noticeable in a good share of the specimens, as noted by Professor Murrill in commenting upon Professor Eaton's statement to the effect "veins are everywhere free," a character distinguishing all true Asplenia. Its propagation through proliferation at the apex, questioned by Professor Murrill, was vouched for[16] in cultivated plants as early as 1878 by Mr. Davenport, and lately Mr. William Palmer has collected fronds in Maryland in which the pinnae bear at their tips minute but well-formed young plants. A good proportion of the plants collected this summer at Havana by Mr. Pollard and myself bear young plants at the apex of the fronds, but I have yet to notice any of fair size and large enough to thrive if separated from the parent plant. I am inclined to believe that these young plants never come to an independent existence. If they are indeed abortive, the fact may be taken as tending to substantiate the claim for its intermediate position.

Another feature in the morphology of the plant which seems especially significant is its marked asymmetry. Even in examining the great growth at Havana it is extremely difficult to discover what would

[14] Ferns of N. Am. 1 : 26.

[15] Fern Bull. 5 : 1. 1897.

[16] Bull. Torr. Bot. Club 6 : 200. 1878.

in an ordinary species be designated as a "perfect frond." Something of this tendency is occasionally seen in *Asplenium pinnatifidum*, but in *A. ebenoides* it is at once the most noticeable and constant feature. In its ordinary mature development it is as constant in this irregularity of shape as most ferns are in their symmetry; this in itself indicates an unusual phylogeny and is a strong bit of evidence supporting its claim to hybridity. A like trait has been noted[17] by Mr. Davenport in the case of his *Dryopteris cristata* × *marginalis*, which, it may be added, Miss Slosson's recent experiments bid fair to prove a true hybrid.[18]

It may be noted further that at Havana *A. ebenoides* in its habitat resembles neither of its supposed parents, both of which require considerable humus and grow mostly upon rocks. *A. ebenoides*, however, is here strictly a mural fern, and like *A. pinnatifidum* and *Pellaea atropurpurea* grows in the narrow chinks of rock where there is a minimum of earth. It is for the most part well shaded and must derive most of its moisture from the porous conglomerate cliff, on the under side of which it grows. The cause of its abundance here is undoubtedly to be found in the especially favorable environment, and perhaps a greater fertility of spores than in the other stations. That it has not a wider distribution in the general region is no more remarkable than the limited distribution of other species, and is perhaps due to a dearth of suitable situations. But Professor Murrill's observations upon the plants found by him in six or seven different localities near Blacksburg, Virginia, show that the fern does well in light rich soil; while a recent letter from Dr. Rusby states that the plants found by him[19] near Newton, N. J., grew in an open sunny spot on a dry limestone ridge; so that after all its habitat is variable. Whenever *A. ebenoides* has been found elsewhere than at Havana it has usually, if not always, been a plant or two in a place, and these rarely in the same general region. What is there to account for this isolation? They might arise from wind-blown spores; but in this event is it not a singular coincidence that the fortunate spore happens on each occasion to settle between plants of *Asplenium platyneuron* and *Camptosorus?* Besides, the stations are mostly separated by considerable distances, one in Vermont, another in Connecticut, in New York, and so on. Thus, though it be a possible, we may hardly regard it as a probable means of origin. Is it not then somewhat plausible to suppose a local origin for the fern at

[17] Papers Pres. Bost. Meet. (1898), 10. 1899.

[18] Fernwort papers. (Ined.)

[19] Bull. Torr. Bot. Club 7: 29. 1880.

each of its few stations by means of a natural hybridization? The rarity of its occurrence is a matter of fact; and, granting the hybridity supposition, it is evident that from the resulting plant of a single cross excessive multiplication is hardly to be expected, be the spores ever so fertile. Evidently much would depend on the environment, and it is easy to assume that the Havana station has proven most favorable, the Virginia locality rather less so, and the remaining stations barely capable of supporting their foundlings. In view of anything like conclusive evidence at the present time it is obviously impossible to settle the contention; my effort has been merely to bring together the related facts known, and to suggest again the possibility or perhaps the probability of such a solution.

It is evident, as has been both affirmed and denied, that the burden of proof rests with those who claim hybridity for the fern; it remains for the sponsors for that theory to prove their contention by actual demonstration. It can be done only by painstaking artificial crossing of the supposed parents; and I am inclined to the belief that this is possible. It is easier, admittedly, to proclaim the hybridity of the fern a myth, its abundance at Havana sufficient evidence that it is a distinct species, the scattering plants mere relics of a more general northerly distribution, and all else mere speculation; but it appears to me that the invariable presence of both supposed parents, the anomalous appearance and peculiar morphology of the fern, its usual occurrence singly and in this connection its very rarity embrace a suggestion of hybridity too patent to be ignored, and of sufficient interest to warrant careful cultural experiments.— WILLIAM R. MAXON, *U. S. National Museum, Washington, D. C.*

CURRENT LITERATURE.

BOOK REVIEWS.

Elements of paleobotany.[1]

THE great needs of recent years in paleobotany have been a summary of the scattered materials and the delimitation of well-founded data from those which are more or less uncertain. A great stride forward has been taken along these lines and as a result we are in a position to speak more categorically as to plant fossils. The first part of Professor Seward's work appeared some time ago and has been reviewed in these pages.[2] Almost simultaneously three valuable works have recently appeared, one in English by Professor Scott,[3] one in German by Potonié, and the one which is the subject of this review.[4] The standpoint of the three works is somewhat different, Scott taking more the standpoint of the morphologist, Potonié of the stratigrapher, while Zeiller combines the botanical and geological standpoints, though giving more emphasis to the botanical side. More than any book that has yet appeared, this is a book to be used with impunity by general readers and elementary students. The first chapter treats of the various methods by which plant fossils have been preserved, then follows a chapter on classification and nomenclature. The body of the book, of course, is made up of descriptions of the various fossil forms treated in order. The cuts are simple but clear and good, and the descriptions are doubtless the shortest and clearest that are found anywhere.

The conservatism of the author is shown at many points, and the difference between established and hypothetical data is clearly brought out. As an illustration of this, Zeiller constantly distinguishes between forms based on leaves and forms based on reproductive organs, as in the ferns. There are interesting discussions of the Sphenophylleæ and the Cycadofilices, though the author does not go so far as some in erecting these forms into great groups by themselves.

At the close of the book are two chapters of extreme interest. The chapter on the succession of floras and climates is wonderfully graphic, and it is doubtful if a better summary of the known facts was ever written, certainly not in a shorter compass. The author theorizes but little from the

[1] This review also appears in the *Journal of Geology*.

[2] Bot. Gaz. **26**: 59. 1898. [3] Bot. Gaz. **30**: 352. 1900.

[4] ZEILLER, R.: Éléments de Paléobotanique. 8vo. pp. 421, with 210 illustrations, Paris: Georges Carré et C. Naud. 1900.

facts presented, and such deductions as he makes in regard to climate are conservative. The last chapter will be somewhat startling to many readers, as Zeiller thinks there is very little evidence from fossil plants in favor of gradual evolution. He states that in almost every case, species, genera, families, and groups appear highly specialized and in their permanent form from the first. So-called intermediate forms like Cheirostrobus appear long after the forms they are supposed to connect. Genera and species that vary now have always varied, and the limits of variation now and in the past have been the same and definitely prescribed. In short, Zeiller believes that the evolution of all groups is a matter almost purely of speculation. Doubtless most botanists will fail to accept Zeiller's views as to evolution, and yet it may be well to put a brake now and then to unlimited speculation; a perusal of Zeiller's final chapter certainly compels one to do that.—H. C. COWLES.

Plant diseases.

IN 1882 the first edition of Robert Hartig's *Lehrbuch der Baumkrankheiten* appeared. This book met with instant favor and was at once recognized as a standard reference work for diseases of trees, especially those caused by the higher fungi. In 1889 the second edition appeared and the favorable reception accorded the first edition was repeated. The third edition has now been issued — this time, however, with a changed title.[5] The change of name from *Baum-* to *Pflanzenkrankheiten* would naturally lead one to expect that the discussion of the subject had been extended so as to include non-woody plants not considered in the previous editions. This is not the case, however, for practically the same plants are treated in the edition before us as in the others. The work is still confined in the main to diseases of woody plants. This is shown by the fact that of the 280 figures only eleven illustrate diseases or parasitic fungi of non-woody plants. Thirty-one pages are given to the discussion of the rusts affecting woody plants and a little over two pages to those affecting non-woody plants. Aside from about six pages given to smuts and short references under *Claviceps purpurea*, *Cystopus candidus*, *Plasmodiophora brassicae*, and the bacterial diseases of hyacinths and potato, the other notes on diseases of non-woody plants caused by fungi are only incidental. The book is divided into five main headings, viz.: (1) injuries caused by plants; (2) diseases caused by atmospheric influences; (3) diseases caused by the action of injurious substances; (4) diseases due to soil conditions; (5) wounds. With the exception of one or two paragraphs, however, consideration is given under the last four heads to woody plants only.

[5] HARTIG, ROBERT: Lehrbuch der Pflanzenkrankheiten. Für Botaniker, Forstleute, Landwirthe, und Gärtner. Dritte, völlig neu bearbeitete Auflage des Lehrbuches der Baumkrankheiten. 8vo. pp. ix + 324. *figs. 280. pl. 1* (colored). Berlin: Julius Springer. 1900. *M* 10.

As compared with the second edition, a somewhat fuller discussion is given to diseases caused by fungi as a whole, and particularly to Ustilagineæ and Uredineæ. The injuries caused by lightning are much more extensively treated than in the previous edition, being given over fourteen pages and twenty-six figures instead of two pages and no figures. The number of figures in the book as a whole has been more than doubled, many being reproductions of photographs to show the habit of certain diseases, while others are detailed drawings made by the author. The colored plate which appeared in the first edition has been retained. In view of the great activity shown in recent years in the study and description of bacterial diseases of plants it seems peculiar to find only two pages devoted to this important subject. Practically the same introductory remarks are given as in the second edition, to the effect that bacterial diseases of plants are not and cannot be numerous in the nature of things, owing to the firm cellulose walls and acid sap characteristic of most plants. But five bacterial diseases are mentioned, two being considered as not yet proved. They are the yellow rot of the hyacinth, the wet rot of the potato, pear blight, and more doubtfully sorghum blight, and the twig gall of the olive. It seems strange that no mention is made of several other economically very important bacterial diseases which have been so carefully and fully described by European and American investigators. A very noticeable feature is the fact that except for a few citations in the introduction to general works, all the publications cited are those by the author himself. He explains this as being done to make these publications more available, since they are scattered through numerous periodicals, etc. There is no need, he believes, for a general bibliography in such a work.

The typography is good, and the figures are mostly excellent. The only typographical error noted is in the legend to *figure 123*, where the name Cryptospora is printed instead of Calyptospora. As a whole the book is likely to prove valuable, not as a general work, however, but rather as a text-book of diseases of trees.—ERNST A. BESSEY.

MINOR NOTICES.

HERMAN B. DORNER (Proc. Ind. Acad. Sci. **1899**: 116–129) has investigated the resin ducts and strengthening cells of the leaves of six species of Abies, and five of Picea, and has discovered differences which enable him to distinguish them. The paper contains text cuts showing the varying structures.—J. M. C.

THE LAST NUMBER of the *Icones Florae Japonicae* bears the date September 1, 1900, and is no. 8 of the spermatophyte and pteridophyte series, edited by T. Makino of the Imperial University at Tōkyō. The present number contains illustrations of species of Davallia, Aldrovanda, Stigmatodactylus, and Saccolabium.—J. M. C.

THE FIFTH FASCICLE of Engler's work on the genera and families of African plants[6] has just appeared, containing Professor Schumann's presentation of the Sterculiaceæ. The genera are 16 in number, and the species 211, the large genus being Hermannia with 73 species. The appearance of these monographs is sumptuous, and the plates are beyond praise.—J. M. C.

A NOTICE of the appearance of the first fascicle of Halácsy's *Flora of Greece* was published in this journal for April last (**29**: 290. 1900). In that notice the occasion for the work and its general character were set forth. The second fascicle[7] has now appeared, and completing Alsinaceæ continues to the beginning of Crassulaceæ. The sequence is that of Bentham and Hooker.—J. M. C.

THE FIRST REPORT of the Michigan Academy of Science has appeared. Its numerous papers indicate a strong and promising organization. The botanical contributions, aside from some general papers by Professor Beal, are as follows: The flora of Michigan lakes, and Teratological forms of *Trillium grandiflorum*, besides several abstracts, by CHARLES A. DAVIS; On saprophytic fungi in the vicinity of the Agricultural College, by B. O. LONGYEAR.—J. M. C.

MR. G. E. STONE, of the Massachusetts Agricultural College, has published a list of the plants of Lake Quinsigamond, a lake near Worcester, and one of the largest in Massachusetts. The flora is evidently one of great interest, and includes some plants which are restricted in central Massachusetts to this lake and its tributaries. The summary shows 88 spermatophytes, 4 pteridophytes, 15 bryophytes, and 331 thallophytes (319 of which are algæ), making 450 species in all.—J. M. C.

ALEXANDER W. EVANS (Proc. Wash. Acad. Sci. **2**: 287–314. *pls. 16–18*. 1900) has just published a paper on the Hepaticae collected in Alaska by the Harriman expedition. There were collected sixty-three species in a condition to be identified, thirty-eight of which are recorded from Alaska for the first time. There are known in Alaska at present eighty-two species of Hepaticae, sixty-seven of which belong to the Jungermanniales. No representatives of the Ricciaceae or of the Anthocerotales are as yet definitely known from the territory.—J. M. C.

LESTER F. WARD (Amer. Jour, Sci. **10**: 327–345. *pls. 2–4*. 1900) has described a number of new species belonging to his genus Cycadeoidea. These cycadean trunks are from the Black hills.. The described species

[6] ENGLER, A.: Monographien afrikanischer Pflanzenfamilien und -Gattungen. V. Sterculiaceæ, bearbeitet von K. Schumann. 4to. pp. 140. *pls. 16*. Leipzig: Wm. Engelmann. 1900. *M* 30.

[7] HALÁCSY, E. DE.: Conspectus Florae Graecae. Vol. I. fasc. II, pp. 225–576. Leipzig: Wilhelm Engelmann. 1900. *M* 8.

now number twenty-nine, and all of them are represented in the collections of the Yale museum. Professor Ward ventures the opinion that we are only upon the threshold of knowledge in reference to the extinct floras of America, and he voices the hope of botanists by stating that "as we penetrate deeper into the inner structure, it is safe to predict that the results will be such as even the botanists proper cannot afford to ignore."—J. M. C.

DR. SMITH ELY JELLIFFE[8] has published a catalogue of the flora of Long Island. His reason for publishing a single list for an island which possesses numerous local floras "lies in the peculiar features of botanical interest that are caused by the position of the island in relation to the mainland, and the history of the growth of plant life on the Atlantic slope, as shown by the combined evidence of geology and botany on Long Island." The author makes an interesting attempt to relate the present flora to what he calls "geological floras." The following statistics of the flora are given: Thallophytes, 719; Bryophytes, 136; Pteridophytes, 41; Spermatophytes, 1342 (Gymnosperms 14, Monocotyledons 322, Dicotyledons 1006); total number of known species 2238.—J. M. C.

FASCICLES 202 and 203 of Engler and Prantl's *Die natürlichen Pflanzenfamilien* have appeared as a double fascicle, completing the first part of the first volume. It is devoted entirely to Flagellata, by G. Senn. The general structure of these somewhat problematical organisms is dealt with in a very satisfactory way. A summary of the group shows the following statistics: seven orders, twenty families, 128 genera, and about 350 species. One cannot but remark the great preponderance of monotypic genera.

The first supplement to this work has also just been issued, including the new material of the year 1897–8 for volumes II–IV. It contains not only the new genera which have been described, but also references to important additional literature. The continuation of these supplements will be of very great value.—J. M. C.

A. A. HELLER has issued a second edition of his "*Catalogue of North American Plants north of Mexico*, bearing the date of November 10, 1900. The first edition was issued in March 1898, since which time the editor states that about two thousand new plant names have been published. The second edition possesses all of the features desirable in a catalogue of this nature; the page is printed upon one side only, giving space for the insertion of new names; larger type has been used than in the previous edition, and the press work is beyond criticism. Such complete lists are absolutely essential to the taxonomist and to those interested in building up collections. The editor gives the interesting calculation that "at the present rate of activity in taxonomic botany, the year 1905 will see twenty thousand plant names to be

[8] The flora of Long Island. 8vo. pp. xvi + 160. Lancaster, Pa.: The New Era Printing Co. 1899. $1.

listed and many changes in generic limits." The list of names is preceded by five pages of changes in nomenclature, which of course involves the citation of the synonyms.—J. M. C.

THE *Flora of the West Indies*, by Urban,[9] has reached the second fascicle of the second volume. The two previous fascicles were noticed in this journal for April last (**29**: 289. 1900). The new fascicle contains the conclusion of the Cyperaceæ by C. B. Clarke, followed by Urban in a long list of corrections of nomenclature and spelling of generic names in the family more in accordance with modern views. The Acanthaceæ are presented by G. Lindau, 37 genera and 90 species being recognized, the new genera being *Drejerella*, *Ancistranthus*, and *Centrilla*. *Dianthera* is merged under *Justicia*. New species of Lauraceæ and Bromeliaceæ are described by C. Mez. "New or little known Leguminosae" is the title of a series of papers by I. Urban, the first one containing about 30 new species, the majority of which belong to *Caesalpinia* and *Galactia*. The new genera are *Hebestigma* (founded on *Robinia? Cubensis* HBK.) and *Rhodopis* (founded on *Erythrina planisiliqua* L.).—J. M. C.

THE PUBLICATION of a state flora does not mean very much in these days unless it devotes considerable attention to the ecological standpoint. The new state flora of Indiana[10] is particularly welcome because of the full ecological as well as geographical notes in connection with each species. Instead of being a mere list, valuable only to the collector, there is material of value to all botanists. In the introduction the physiographic features, climate, and soils are described briefly, and then the author discusses the plant societies. After a statement of general principles, the hydrophytes, xerophytes, and mesophytes are treated in order, especial attention being given to the xerophytes. Topics of more general economic interest come next, notably reforestation, poisonous plants, and weeds. A very complete bibliography closes the introduction, and the remainder of the book (400 pp.) gives a detailed account of the various species. Through an unfortunate oversight the reprint is not dated, nor is there any indication as to its source. —H. C. COWLES.

T. C. PALMER and F. J. KEELEY (Proc. Acad. Nat. Sci. Philad. **1900**: 465–479. *pls. 15, 16*) have published concerning the structure of the diatom girdle. They have noticed that in all literature upon diatoms there is a tendency to neglect the structure of the girdle and to take for granted that it

[9] URBAN, IGNATIUS: Symbolae Antillanae seu fundamenta florae Indae occidentalis. Vol. II, fasc. 2, pp. 161–335. Berlin: Gebrüder Borntraeger. 1900. *M* 9.90.

[10] COULTER, STANLEY: A catalogue of the flowering plants and of the ferns and their allies indigenous to Indiana. Separate reprint from Report of the Indiana State Geologist, 1899, pp. 553–1074.

is everywhere the same, and that it is a typically closed hoop. Their present claim is that the closed hoop structure is unusual. They make the statement that "with some very important exceptions, the girdle is a two-ended band of silica, with the ends variously and characteristically rounded or otherwise modified, and approximated or overlapping without being joined. The position of the gap or joint is, within limits, constant in a given genus with relation to salient features of the valves. In case of each simple pair of primary girdles, the two gaps are usually at opposite points of the diatom; and in general, in the forms we have studied, the gaps are normally so situated with respect to each other as to 'lap joints.'" These claims are supported by the presentation of typical examples.—J. M. C.

NOTES FOR STUDENTS.

THE EFFECT of certain inorganic salts in accelerating the growth of lower plant organisms has recently been made the subject of research by N. Ōno.[11] He has worked with the following substances in very dilute solutions: $ZnSO_4$, $FeSO_4$, $NiSO_4$, $CoSO_4$, $CuSO_4$, $HgCl_2$, $LiNO_3$, NaF, and K_2AsO_3. For fungi *Aspergillus niger* and *Penicillium glaucum* served as subjects; for algæ (and this is the first time this group has been brought under experimentation in this regard), *Protococcus* sp., *Chroococcum* sp., *Hormidium nitens*, and *Stigeoclonium* sp. Suitable nutrient fluids were used unmodified for the control cultures, and with addition of one of the above-named salts for the tests.

With the exception of $CuSO_4$ and $HgCl_2$, all of the salts, in very dilute solution, act upon the algæ to accelerate vegetative growth and to retard the process of zoospore production. All of the salts act in this way upon the fungi studied; in this the author is in accord with Richards.[12] For the algæ the optimum growth is obtained by addition of a much smaller amount of the unusual salt than for the fungi. In the former case the concentration of this compound varies from $\frac{1}{2{,}500{,}000}$ to $\frac{1}{100{,}000}$ gram molecule per liter; in the latter from $\frac{1}{4000}$ to $\frac{1}{800}$ gram molecules per liter. Sixteen pages of tables give the results very clearly. The author has been content to determine the facts as stated above, without inquiring more deeply into their nature. He seems to make the mistake of considering his salts as remaining intact in these dilute solutions, instead of being completely dissociated, as they almost certainly are. It is very probable that (excepting in case of

[11] Ueber die Wachstumsbeschleunigung einiger Algen und Pilze durch chemische Reize. Jour. Coll. Sci. Imp. Univ. Tōkyō. **13**: 141–186. *pl. 13*. 1900.

[12] Die Beeinflussung des Wachsthums einiger Pilze durch chemische Reize. Pringsh. Jahrb. f. wiss. Bot. **30**: 665. 1897.

the last two salts in our list) the accelerating stimulus is due to the cations, and if this be true it could easily have been demonstrated by the use of equimolecular solutions of different salts of the same metal. That the effect is not due to the anions we must conclude from the fact that the control solutions contains SO_4-ions from $MgSO_4$, and NO_3-ions from NH_4NO_3 and KNO_3. It may be profitable to call attention to the fact that where NaF and K_2AsO_3 are used the stimulus to growth must be due not to the cations but to the anions. However, the material at hand is far too meager for any general conclusions along this line.

If our author has seemed somewhat easily satisfied as to the chemical nature of the stimulus with which he is dealing, the omission is palliated by a decided advance which he has made in another line. Heretofore experiments upon acceleration and retardation of growth have gone no further than to determine the relative increase in somatic material. Now, since of the materials taken into the plant body some are used to increase its mass and others go out again more or less completely oxidized, thus contributing energy rather than matter to the organism, it becomes essential to determine whether or not accelerated growth means also a relative increase in the amount of waste products. Of course in the algæ these products of katabolism take the form mainly of CO_2, and, owing to the photosynthetic process, its amount cannot be easily determined. But in fungi we have no such disturbing factor. Much of the waste from these fungi comes off in the partially oxidized form of oxalic acid. The relative amounts of this excreted in the different cases was determined by titration and compared with the amount of sugar taken from the fluid by the growing plants. Relatively much more acid was given off in the normal cultures than in those containing $ZnSO_4$, $CuSO_4$, $CoSO_4$, $HgCl_2$, and NaF. For example, with $ZnSO_4$, in twenty-five days *Aspergillus niger* gave off per gram of its own weight 0.443^{gm} of acid. Without $ZnSO_4$ the amount given off was 2.245^{gm} per gram of its own weight. $NiSO_4$ was the only exception; it acted in the opposite way. What are called "economic coefficients" are obtained by dividing the amount of sugar used by the amount of substance produced in the plant body. This coefficient is much greater in the control cultures than in the others. They average about as one to two. If the weight of the fungus and that of the sugar absorbed could have been reduced to calories, and then this coefficient found as above, it will be seen that it would have been the reciprocal of what, in mechanics, would be termed the efficiency. Thus the result above enunciated seems to show at least that the efficiency of the fungus, as a machine for storing up material, increases with the addition of traces of these poisons. This seems to open up a new method for obtaining some quantitative measurements of the metabolic process. But since the author did not have it in mind his data are not complete enough for this purpose.

One other point is made in the paper. Loew has shown[13] that the spore-producing function of fungi is much more susceptible to the action of poisons than is the vegetative. Thus it should be possible to obtain a concentration of the poison such that it would hinder or stop spore production and still not effect, or effect in a minimum degree, the vegetative growth. But under these conditions the energy normally directed to spore production would probably be turned towards growth. Thus we would have an acceleration in growth due to the retardation in reproduction. This, the author concludes, is probably the case in his researches.— BURTON EDWARD LIVINGSTON.

ITEMS OF TAXONOMIC INTEREST are as follows: W. N. SUKSDORF (Bot. Monatsschrift **18**: 97–99, 132–134. 1900), in continuing his descriptions of Washington plants, has published new varieties in *Viburnum*, *Valerianella*, *Madia*, *Artemisia*, *Troximon*, *Dodecatheon*, *Phlox*, and *Gilia* (3), and new species of *Dodecatheon*, *Gilia*, and *Amsinckia* (4).—In continuing his studies on the fungus flora of South America, DR. H. REHM (Hedwigia **39**: 209–224. 1900) has reached the Discomycetes, in which he describes *Physmatomyces* (Bulgariaceæ) and *Psorotheciopsis* (Mollisieæ) as new genera.—PASCAL CONTI (Mém. Herb. Boiss. no. **18**: 1–86. 1900) has published a revision of the species of *Matthiola*, recognizing 32 species, 5 of which are described as new.— MARCUS E. JONES (Zoe **5**: 41–53. 1900) has published the ninth of his "Contributions to Western Botany," which contains many critical notes, some new forms of *Astragalus*, and a very interesting ecological sketch of the Great Salt Lake desert.—WILLIAM F. WRIGHT (*ibid.* 53–58) has published seven new species and two new varieties of *Galium*, chiefly from California. —E. J. DURAND (Bull. Torr. Bot. Club **27**: 463–495. *pls. 27–32*. 1900) has published a classification of the fleshy Pezizineæ, segregating them into four families on the basis of the structure of the sterile layers of the cup.—E. J. HILL (*ibid.* 496–505) has published an interesting account of *Celtis pumila* Pursh, which he shows should be restored to specific rank.—GEORGE E. OSTERHOUT (*ibid.* 506–508) has described six new forms from Colorado, under *Allium* (2), *Artemisia* (3), and *Agoseris* (1).—AVEN NELSON (from tenth annual report of the Wyoming Exper. Sta.) has published under the title "The Cryptogams of Wyoming" a list of those species which have been secured in the botanical survey of the state.—E. KOEHNE (Engler's Bot. Jahrb. **29**: 161–168. 1900) has completed his account of new Lythraceæ.—L. DIELS (*ibid.* 169–320. *pls. 2–5*) has begun the publication of the flora of Central China, a general consideration of the nature of the region covered being followed by a list of species, including new ones, from Filicales to Caryophyllaceæ inclusive. The paper contains descriptions of 9 new Ferns, 26 new Monocotyledons, and 13 new Dicotyledons so far as the group is considered. *Smilax* contains 10 new species, and a conspectus of *Fagus* is

[13] Ein natürliches System von Giftwirkung. 1893.

given, 7 species being recognized, one of which is new.—KARL FRITSCH (*ibid.* Beiblatt 5–23) has published an account of the Gesneriaceæ of Brazil. —OTTO V. SEEMAN (*ibid.* Beiblatt 28) has published two new species of *Salix* (*S. pseudolapponum* and *S. aemulans*) from Colorado, collected and distributed by Baker, Earle, and Tracy.—F. V. COVILLE (Proceedings of the Washington Academy of Science **2**: 275–286. *pl. 15.* 1900) has given an account of the tree willows of Alaska, recognizing five species, one of which (*S. amplifolia*) is described as new.—A. W. EVANS (*ibid.* 287–314. *pls. 16–18*) has published notes on the Hepaticae collected in Alaska by the Harriman Expedition, the list containing sixty-three species.—C. L. POLLARD (Proc. Biol. Soc. Washington **13**: 169. 1900) has described a new violet from Alabama.—E. L. MORRIS (*ibid.* 171–182) has discovered in West Virginia forty-seven species heretofore unreported from the state, and also new subspecies of *Polypodium vulgare* and *Vernonia gigantea.*—JOHN M. HOLZINGER (Asa Gray Bull. **8**: 95–99. *pl. 6.* 1900) has reported from Yellowstone National Park a Polytrichum (*P. Jensenii* Hagen) new to the United States, and heretofore known from northern Europe.—K. M. WIEGAND (Bull. Torr. Bot. Club **27**: 511–527. 1900) has published upon *Juncus tenuis* and some of its North American allies, discussing thirteen species, and describing four species as new.—P. A. RYDBERG (*ibid.* 528–538), in continuing his "Studies on the Rocky mountain flora," presents the species of Melanthaceæ, finding that the family is represented in the region under consideration by at least five genera and seventeen species. Of these, one genus (*Stenanthella*) and seven species are described as new. The new genus is based on *Stenanthium occidentale* Gray, and the new species belong to *Tofieldia* (1), *Veratrum* (1), and *Zygadenus* (5).—W. N. SUKSDORF (Bot. Monats. **18**: 153–156. 1900), in continuing his account of Washington plants, has described new species or vareties of *Pentstemon, Mimulus, Castilleia, Aphyllon,* and *Listera.*—In Mém. Herb. Boiss. (no. 20, October 1900) publication of the African flora is continued, new species being described under numerous families by the various collaborators, and under Scrophulariaceæ STAPF describes and figures a new genus under the name *Dintera.*—J. M. C.

ARNOLDI'S[14] third contribution to the morphology of gymnosperms deals with the embryology of *Cephalotaxus Fortunei.* Systematists have placed Cephalotaxus between Phyllocladus and Ginkgo on the one side and Torreya and Taxus on the other. In anatomical features it closely resembles Taxus, while the structure of its ovule allies it more nearly with Ginkgo and the cycads. It was the object of this study to determine whether embryology would throw any light upon the relationships of this interesting form. The material was obtained from a botanical garden in southern Russia. Alcohol

[14] ARNOLDI, W.: Beiträge zur Morphologie der Gymnospermen, III. Embryogenie von *Cephalotaxus Fortunei.* Flora **82**: 46–63. *pls. 1–3.* 1900.

was used for fixing, and Zimmermann's fuchsin and iodine green method for staining.

As in many other gymnosperms, the ovules require two years for ripening. In the first year the nucellus and integument are formed and pollination takes place. In the spring of the second year the archesporium and embryo-sac are formed; endosperm appears in May, and before the end of the month the archegonia are developed; fertilization occurs about the middle of June, and during August the embryo is fully formed.

The principal results of the study are as follows:

1. The neck of the archegonium consists of two cells.

2. The material for the growth of the egg is supplied by the nuclei of the cells immediately surrounding it, and has at first the form of very small droplets; after these have passed into the egg they attain considerable size and some complexity of structure; and serve as nourishment for the embryo in its earliest stages.

3. The nucleus of the central cell divides, but does not form a ventral canal cell. The upper part of the central cell with its nucleus then becomes mucilaginous, destroys the neck cells, and presses out from the egg.

4. The pollen tube contains four nuclei, the two male cells being of the same size.

5. After the pollen tube has discharged its contents, one of the male nuclei fuses with the egg nucleus, while the other remains in the upper part of the egg where it may divide amitotically. The fusion nucleus moves to the middle of the egg and divides three or four times. The daughter nuclei divide at the bottom of the archegonium and thus there arises a number of free cells. These cells then become arranged into tiers in much the same manner as in Taxus, according to Jaeger's recent description. The later stages in the development were described long ago by Strasburger.—C. J. CHAMBERLAIN.

THE LIFE HISTORY of a new and interesting member of the Chytridiales has been described by Gobi.[15] This form, *Rhizidiomyces ichneumon*, is found on one of the Volvocaceæ, *Chloromonas globulosa*, and may multiply so rapidly as to create an epidemic among these unicellular organisms. The swarm spores of the fungus settle down on the surface of the Chloromonas, both the motile and resting cells, and there put forth a process which enters the host. While most of the protoplasm of the parasite remains outside of the algal cell, a connection becomes established with the host by the enlargement of the entering process into a sort of bulb on the inside, and the development from this of several filamentous outgrowths. These latter push

[15] Ueber einen neuen parasitischen Pilz, *Rhizidiomyces ichneumon*, nov. sp., und seinen Nährorganismus, *Chloromonas globulosa* (Perty). Scriptis Botanicis Horti Univ. Imp. Petropolitanae **15**: 251–292. *pls.* 6–7. 1899.

into the Chloromonas cell, which eventually dies. The amount of protoplasm in the Rhizidiomyces increases rapidly, until the portion outside of the algal cell is almost as large as the host itself. This part then prepares to develop and discharge the swarm spores. The protoplasm is gathered from the interior, and a neck is formed through which the divided contents escape into the water as small uniciliate zoospores that swim slowly away.—B. M. DAVIS.

GOBI[16] has investigated the life history of a new species of Pythium that lives in the dead and dying filaments of Vaucheria. *Pythium tenue* is a very delicate form whose hyphae can hardly be more than 1–3μ in diameter. It grows in the interior of the Vaucheria filaments, sending an occasional free end of the mycelium through the wall of the alga to the exterior. The end of such a hypha becomes swollen, but is not cut off by a cross wall as is usually the case when sporangia are formed. However, the protoplasm in its interior develops several swarm spores which shortly escape. The swarm spores quickly germinate, coming to rest in the exterior of the Vaucheria filament, where each puts forth a germ tube that pierces the cell wall and within three hours may develop a long hypha. The sexual organs are small and chiefly interesting from the fact that the end of the antheridial filament is not cut off as a distinct cell. The oospore is 8–9μ in diameter and possesses a smooth wall. Nothing is known of its cytoplasmic structure, and its germination has not been observed.—B. M. DAVIS.

MR. O. F. COOK has published in *Science* (**12**: 475–481. 1900) his very interesting paper on "The method of types in botanical nomenclature." After a well organized discussion of the subject, the following general statements are advanced. "In biology a species is a coherent or continuous group of organisms." "For nomenclatorial purposes a species is a group of individuals which has been designated by a scientific (preferably a Latin adjective) name, the first individual to which the name was applied constituting the type of the species." "For purposes of reference and citation specific names which appeared previous to the *Species Plantarum* of Linnaeus are not regarded in botanical nomenclature." "A genus of organisms is a species without close affinities, or a group of mutually related species." "A generic name is established in taxonomy when it has been applied to a recognizable species." "The generic taxonomy of plants may be treated as beginning with Tournefort's *Institutiones* (1700)."—J. M. C.

MR. L. A. BOODLE has begun the publication of a series of papers on the comparative anatomy of the Hymenophyllaceae, Schizaeaceae, and Gleicheniaceae, the first[17] dealing with the Hymenophylleaceæ. A summary

[16] GOBI: Entwickelungsgeschichte des *Pythium tenue*, nov. sp. Scriptis Bot. Horti Univ. Imp. Petropol. **15**: 211. *pls. 4–5.* 1899.

[17] Annals of Botany **14**: 455–496. *pls. 25–27.* 1900.

of his results is as follows: the stem is monostelic, and one leaf trace passes to each leaf; the stele contains no pith, and is of several types, (1) a xylem mass with internal protoxylem, connected with leaf traces, (2) a xylem mass with indefinite scattered protoxylem, (3) a xylem mass with peripheral protoxylem, (4) sub-collateral, (5) a collateral bundle. The author thinks that the sub-collateral structure has probably been derived from a more complicated type by reduction, and that the filmy habit of Hymenophyllum is probably not primitive.—J. M. C.

MR. R. F. SHOVE has published[18] an account of the structure of the stem of *Angiopteris evecta*, which in 1864 had been described in detail by Mettenius, but from whom the present author differs in several particulars. The position of the protophloem in the stem stele is said to be anomalous, occurring on the outer side of the stele in the form of a discontinuous arc. The centrifugal growth of the phloem is also said to be contrary to that described for most other ferns, but the other Marattiaceæ are not included in this comparison. The mesarch and endarch structure of the steles was confirmed, and also the presence of several initials at the apex of the stem. – J. M. C.

W. L. BRAY discusses the relations of the North American flora to that of South America.[19] Few new conclusions are reached, but there is an interesting summary of the known data on the subject. The most important agencies which have brought about relationships are those which have acted in the past; the endemic northern floras of Mexico and the Andes are to be referred to such causes. Minor agencies of distribution are still active, especially those along the gulf zone and those due to man.—H. C. COWLES.

ULE DESCRIBES several isolated observations[20] that he has made concerning the influence of animals upon plants in the tropics. These observations have to do mainly with pollination and seed dispersal. He opposes the view that palms and other plants which develop a considerable amount of heat in the flowers do so with any relation to pollination. This theory is *a priori* improbable, and the author adds that no protection would be necessary to the insects in tropical climates, and furthermore that the insects do not fly at night.—H. C. COWLES.

MISS ETHEL N. THOMAS has announced[21] the discovery of double fertilization in *Caltha palustris*. The original discoveries of this phenomenon, it will be remembered, were among the Liliaceæ, so that the discovery of

[18] Annals of Botany **14**: 497–525. *pls. 28–29*. 1900.

[19] Science **12**: 709–716. 1900.

[20] Ber. deutsch. Bot. Ges. **18**: 122–130. 1900.

[21] Annals of Botany **14**: 527–535. *pl. 30*. 1900.

Miss Thomas was interesting in extending the phenomenon to the dicotyledons. Before the publication of her paper, however, Nawaschin had announced his discovery of double fertilization in Helianthus and Rudbeckia, and its probable occurrence in Delphinium; while more recently Land[22] has included Erigeron and Silphium.—J. M. C.

W. H. LONG, JR.,[23] gives an interesting preliminary account of the ecological distribution of fungi in the vicinity of Austin, Texas. In general the conditions do not seem to favor a great abundance of fungi, the numbers being limited by the nature of the soil, the paucity of forests, and the climate. Parasitic forms are limited by reason of the xerophytic nature of the host leaves. The saprophytic forms are ecologically subdivided into dung inhabiting species, cedar brake fungi, post oak land species, species of grassy places, epixylous species, and species of open rocky soil.—H. C. COWLES.

MR. JAMES BRITTEN has done good service to taxonomists in publishing in the *Journal of Botany* (**38**: 430–443. 1900) a textual reproduction of the two pages of Linnaeus's *Systema Naturae*, ed. i, which give in tabular form the classification of plants. The occasion for this is the great rarity of the work, which is a folio, whose preface is dated from Leyden, July 23, 1735.—J. M. C.

DR. HERMANN VON SCHRENK has published an account[24] of two diseases of red cedar, one called "white rot," and caused by *Polyporus juniperinus*, described as a new species; the other called "red rot" or "pecky cedar," and caused by *Polyporus carneus*. Seven plates, mostly from photographs of diseased wood, accompany the paper.—J. M. C.

THE CALYX HYDATHODES which have been found by Koorders are confined to tropical plants. Shibata[25] adds five Japan species, and finds that the hydathodes secrete mainly water, but that they also secrete some sugar and thus have a relationship to nectaries.—H. C. COWLES.

[22] Botanical Gazette **30**: 252–260. 1900.

[23] Bull. Torr. Bot. Club **27**: 579–588. 1900.

[24] Bulletin 21, U. S. Dept. of Agric. Div. of Veg. Phys. and Path. 1900.

[25] Bot. Centralbl. **83**: 350. 1900.

NEWS.

DR. DAVID GRIFFITHS has been appointed professor of botany in the University of Arizona, and also botanist to the Experiment Station.

DR. E. PALLA, *privat-docent* in the University of Graz, left home at the end of September for a long anticipated sojourn in Buitenzorg, Java.

DR. M. VON RACIBORSKI has been appointed professor of botany and director of the botanical gardens in the agricultural school at Dublaney, near Lemberg.

A BOTANICAL SCHOOL is being erected at a cost of $20,000 in Schenley Park, Pittsburgh, being intended especially for teachers and classes from the public schools.

PROFESSOR L. M. UNDERWOOD spent the summer in studying American ferns in the British Museum, in the Kew Gardens, and in the Cosson Herbarium in Paris.

DR. N. L. BRITTON represented the New York Botanical Garden at the recent International Botanical Congress at Paris, and was also an accredited delegate from the U. S. Government.

OF THE THIRTY names selected to represent America's greatest men in the Hall of Fame of the University of New York only two are those of naturalists. One of these is Asa Gray and the other J. J. Audubon.

WE LEARN from *Science* that the Yale Forestry School has opened with an enrollment of seven regular students and seventeen from other departments, the residence of the late Professor O. C. Marsh being used as a school building. The degree of Master in Forestry will be given to such students of the school as have previously received the bachelor's degree from collegiate institutions of high standing.

MUHLENBERGIA is the title of a new journal of botany, whose initial number is dated November 10, and whose editor and publisher is Mr. A. A. Heller. The publication office is Lancaster, Pa., and the subscription price is $1.00 a volume, the volume to contain not less than 150 pages. The journal is to be issued at irregular intervals, and the first number (8 pp.) consists of a list of changes in nomenclature by the editor.

THE *Journal* of the New York Botanical Garden is designed to be chiefly an organ of communication with the members of the Garden organization (now numbering about a thousand), who are thus to be kept informed of

the progress and development of the garden in all its departments and of the activities of people connected with it in any way. At present technical articles will not be published, though botanical notes, news, and reviews will often be included.

THE *O. S. U. Naturalist* is the title of a new journal whose first number appeared in November. It is a journal devoted "more especially to the natural history of Ohio," and is the official organ of the Biological Club of the Ohio State University. It is published monthly during the academic year, and its subscription price is fifty cents. The editor-in-chief is Professor John H. Schaffner, of the University. The first number contains two botanical papers by Professor W. A. Kellerman.

IN THE *Bulletin* of the Torrey Botanical Club for October, some of the papers presented at the celebration of "Torrey Day" in connection with the last meeting of the A. A. A. S. are published. Dr. N. L. Britton has written an exceedingly interesting sketch of Dr. Torrey as a botanist, and follows it by a bibliography; Dr. Edward S. Burgess has presented the work of the Torrey Botanical Club; while Dr. James Hyatt, one of the earliest members of the club, Professor T. C. Porter, and Professor Charles H. Peck, have put in permanent form some delightful personal reminiscences of Dr. Torrey.

BY THE WILL of the late Charles H. Smith, of Providence, R. I., the botanical department of Brown University receives $2,000 the income from which is to be expended under the approval of the senior professor of botany, now Professor W. W. Bailey. The city of Providence is made residuary legatee for the development of parks. The income, which is likely to amount to ten or twelve thousand dollars annually, is to be applied to the purchase of plants for the parks, greenhouses, etc., under the advice and direction of the superintendent of parks, and the senior professor of botany of Brown University. One thousand dollars is bequeathed to the city as a trust fund, the income to be used for purchasing microscopes for the high schools.

MR. B. T. GALLOWAY has been appointed Director of Plant Industry, Mr. Albert F. Woods has been promoted to be Chief and Mr. M. B. Waite Assistant Chief of the Division of Vegetable Physiology and Pathology, U. S. Department of Agriculture. The Secretary has issued the following general order: "For the purpose of unifying the work of certain branches of the department, it is hereby ordered that the Chief of the Division of Vegetable Physiology and Pathology, the Chief of the Division of Agrostology, and the Chief of the Division of Pomology confer upon all matters of general policy and plan with the Superintendent of Experimental Gardens and Grounds, who is hereby designated as Director of Plant Industry. In carrying out this order the several branches of the department named will maintain their integrity and organization."

NOW THAT the *Pflanzenfamilien* is about complete, the energetic editor, Dr. Adolph Engler, has entered upon an even more gigantic undertaking. The publisher, William Engelmann, announces the beginning of a work, *Das Pflanzenreich*, which is to be a conspectus of the plant kingdom, *i. e.*, a critical enumeration of all orders, families, genera, sub-genera, species, and varieties. It is proposed to issue the work in separately paged parts, each family being complete in itself, with its own index, though smaller families will be united in parts containing two to four signatures. The arrangement of the matter will be as follows. At the head of each family will be given its literature, including both systematic and general works. Purely systematic monographs of genera, however, will be listed under the appropriate genus. The general sections — vegetative organs, anatomical relations, etc.—will be written in German and briefer than in the *Pflanzenfamilien;* the diagnoses of families, genera, sections, and the enumeration of species will be in Latin. Only important synonyms (with the year), and only really good characteristic illustrations will be cited. Exsiccatae may also be cited in doubtful cases. The style of citation will be uniform. Thirty collaborators have agreed upon the nomenclature to be used. It must not be supposed that this is merely a revision of the *Pflanzenfamilien*. That work will be kept up to date by biennial supplements, and the new one, beginning with the families most in need of new elaboration will proceed on its own lines. Twenty years, it is anticipated, will be required for its completion. The support, scientific and financial, of the Prussian *Kultusministerium* and the Prussian Academy of Sciences, together with the proved executive ability of editor and publisher, are strong guarantees that the work will progress to a satisfactory completion.

GENERAL INDEX.

The most important classified entries will be found under Contributors, Personals, and Reviews. New names and names of new genera, species, and varieties, are printed in **bold-face type**; synonyms in *italics*.

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